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The effect of a methionine deficient or balanced diet on several metabolic responses in turkey poults

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by

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INTRODUCTION

In the past two decades, poultry production has been expanded rapidly to meet human food needs. This has been possible because of the high degree of efficiency developed in the broiler industry. Contributions to this efficient production are due to the advanced technology of nutrition, breeding and management in poultry. The majority of research in nutrition has been focused on practical aspects.

Basic studies, such as the effect of nutritional status on metabolic regulation or responses, should be as equally emphasized as the practical aspects because much is yet to be learned which may later lead to practical applications. For instance, that a very small amount of crystalline amino acids supplemented into some diets can promote a vigorous growth rate is no longer a mystery, since we now know animals are fed to meet essential amino acid requirements rather than protein. Therefore, while the practical technology satisfies current conditions, basic studies could provide a good guide for the future.

Furthermore, basic studies in poultry nutrition can provide information for improving human health. For example, there is a high incidence of atherosclerosis in poultry. Since it is not practical to do fundamental studies on human heart and aorta, cockerels have become a favorable experimental animal to provide knowledge for combating this fatal

disease in humans.

For these reasons, we conducted three experiments to investigate the effect of protein levels and limiting amino acis(s) supplementation on metabolic responses in male turkey poults. There are three unique characteristics of these studies: (1) Instead of using a wide range of protein levels (such as feeding a nitrogen-free diet versus a high-protein diet) we kept protein levels within a normal range. (2) In all three experiments, we used male turkey poults which were fast growing and very sensitive to nutritional status. (3) The period of the experiments was from 1 to 7 weeks of age while most basic studies with poultry have been carried 1 to 2 weeks of age. Although very short-range (2 weeks or less) amino acid studies contribute much information which is applicable to the birds' early life, the age effect on amino acid metabolism is very critical in this growing period and the later amino acid studies cannot be ignored.

Finally, it was our sincere hope that these studies would contribute to the general knowledge of protein or amino acid(s) metabolism in other species as well as in poultry.

REVIEW OF LITERATURE

Dietary Protein and Amino Acid Supplementation

As regards dietary protein level and amino acid balance studies in turkey poults, a considerable amount of excellent research has been conducted in the Poultry Science Department at Iowa State University. However, most of these experiments have been concentrated on the practical aspects — body weight gain and feed efficiency.

Heady, Balloun and Dean (1956) and Balloun and Phillips (1957) reported a protein level of 26 to 27 percent as optimum for poults for the first six weeks of age.

Balloun et al. (1959) studied energy and protein requirements for turkey starting diets. They concluded that the chief effect of increasing protein was greater weight gains, while increasing energy usually increased growth and nearly always improved feed efficiency. The ratio of protein to energy was of relatively minor importance as compared to simple protein and energy effects.

Balloun (1962) investigated lysine, arginine and methionine balance of diets for turkeys to 24 weeks of age. Methionine tended to improve weight gains to 6 weeks of age when a 28% protein corn-soybean meal diet was fed. Arginine supplementation improved weight only a small amount.

In addition, Balloun (1967) reported in young turkeys (1

to 40 days of age) that as good performance can be obtained from 24% or 26% corn-soybean meal diets as from the 28-30% diet usually fed if the low-protein diets are supplemented with methionine and lysine. In these trials, when the protein was further reduced to 22% by replacing soybean meal with corn, the addition of four amino acids (methionine, lysine, glycine and arginine) improved weight gains of the poults 27% over gains made by poults fed the unsupplemented 22% protein diet. The weights achieved, however, were still 10% less than those made by poults fed the amino acid-supplemented 24% and 26% protein diets.

Askelson and Balloun (1965) demonstrated with broiler chicks that monosodium glutamate or L-glutamic acid supplementation of diets to increase the crude protein content from 18 to 22 percent had no effect on chick weight and feed efficiency.

Keshavarz and Fuller (1971) studied the relationship of arginine and methionine in chicks and the significance of creatine biosynthesis in their interaction. They reported that adding graded levels of arginine to a corn-soya diet limiting in methionine caused a growth depression in chicks corresponding to the levels of supplemental arginine, which could be alleviated by adding small quantities of methionine. The growth depression created by arginine supplementation was accentuated in the presence of additional glycine or alpha-

aminoisobutyric acid. Betaine was similar to methionine in alleviating the adverse effect of added arginine. The supplemental arginine increased creatine in muscle and excreta, indicating that at least one of the mechanisms involved in the arginine-methionine interaction is the formation of creatine.

Proteolytic Activity of Pancreas

The reports of Grossman et al. (1943,1944) indicated that the synthesis of enzymes by the pancreas was affected by the nature of the diet. Ben Abdeljlil et al. (1963) reported an increased secretion of trypsinogen and chymotrypsinogen by the pancreas of rats fed a 70% casein diet. High protein, low starch diets have been reported by Howard and Yudkin (1963) to result in greater protease synthesis by the rat pancreas.

Imondi and Bird (1967) studied the effects of dietary protein level on chick growth and on proteolytic activity of the avian pancreas. They reported that age or size of the chicks, or both, may influence pancreas size; however, the pancreas size remained relatively constant when expressed as a percentage of body weight. The mitotic rate measurements indicated that the growth of the pancreas of normal chicks was due primarily to cellular hypertrophy. In the protein-depleted chicks, the pancreatic growth observed after feeding

protein-containing diets appeared to result from hyperplasia. For proteolytic enzyme activity of the pancrease, irregular results were observed; however, a statement to the effect that, with chicks, surfeit protein feeding results in a greater synthesis of pancreatic protease than does the feeding of an inadequate level of protein is probably justifiable.

Homeostatic Effect

In 1965, a review by Nasset on the nutritional significance of endogenous nitrogen secretion in nonruminants pointed out that the presence of this source of nitrogen in the lumen of the gastrointestinal tract acts as a homeostatic device which prevents wide fluctuations in the amino acid mixture available for absorption. He states that the effectiveness of such homeostasis depends probably upon the presence of a large and mobile reserve in the alimentary tract itself. The amount of endogenous nitrogen in the gut lumen is surprisingly great and, depending upon conditions, frequently exceeds the quantity of ingested nitrogen by several fold.

Bird (1968) and Bolton (1964) have shown that the nitrogen in the duodenum is greatly increased from endogenous sources and dilutes the dietary protein. Thus, it may be assumed that in the bird the amino acid mixture in the in-

testinal lumen must be more or less constant irrespective of diet, similar to that described in dogs by Nasset (1965). Factors that affect the amount of endogenous nitrogen content of the small intestine must have an important bearing on amino acid metabolism.

Data presented by Snook and Meyer (1964) indicate that endogenous nitrogen secretions and digestive enzymes markedly increased in response to protein feeding. They also estimated that about 90% of the endogenous nitrogen was digested and absorbed. It would appear that the intraluminal pool of amino acids has some homeostatic advantage because the amino acid mixture available for absorption is complete. Ingestion of an incomplete protein probably results in the body having a temporary amino acid deficit found principally in the digestive glands.

Serum Protein and Albumin

There has been considerable interest in the possible use of serum protein or albumin levels as a sensitive biochemical index for appraising protein nutritional status. Feeding low protein diets has been associated with decreased total serum protein and serum albumin levels in rats by Allison (1955) and in chicks by Leveille et al. (1960) and Leveille and Sauberlich (1961).

According to Arroyave (1962), plasma albumin values are

usually lower in human populations with a low socioeconomic standard of living and with a diet low in protein of good quality. However, Albanese (1959) has cited cases where low protein intakes have not been associated with low plasma protein levels. Graham et al. (1966) found that inadequate caloric intakes of infants, although adversely affecting weight gain, favored the synthesis of serum albumin.

Thomas and Combs (1967) concluded that total serum protein or albumin level may serve as an index of body composition of chicks when the protein of the diet is either adequate or deficient in a single amino acid because there is a very high correlation between either total serum protein or albumin level and body composition.

Bierer (1969) studied serum protein fractions of 10- to 12-week-old female Broad Breasted White turkeys. He demonstrated that 9 fractions were isolated from each of these serum samples, tentatively identified as prealbumin, albumin, alpha 1, alpha 2, alpha 3, beta, gamma 1, gamma 2, and post-gamma. Total protein content of these serum samples varied somewhat with the method of analysis. One spectrophotometric biuret method result of 3.35 gm, and a serum protein meter result of 3.67 gm per 100 ml of serum were obtained.

Liver DNA and RNA

The significance of DNA in nutritional studies is because of the stable characteristic in tissue. DNA is a genetically determined cellular constituent and is affected relatively little by environmental influences. In most species, the liver DNA content per cell and the amount of DNA in the whole liver are not changed by starvation or by protein deficiency.

In contrast, the RNA content of tissues is altered easily by the quantity and quality of the dietary protein. Because the RNA in tissue plays a major role of transcription and translation, the RNA to DNA ratio is often used as an index to measure the magnitude of tissue protein synthesis.

Wannemacher and Allison (1968) have demonstrated that the loss of cellular protein from the liver, muscle, and skin was correlated with a decrease in the RNA to DNA ratio when adult rats were fed a protein-free diet for 100 days.

Squibb (1968) reported that increasing levels of dietary lysine above normal requirement for chicks caused a reduction of RNA and tissue protein, and an increase in DNA due to the starvation effect.

Enwonwu et al. (1971) studied synthesis and degradation of liver ribosomal RNA in fed and fasted rats. They found that (1) synthesis of liver RNA is not depressed by fasting until significant losses of RNA and polysomes have occurred,

(2) the effect of fasting on degradative rate varies with the duration of starvation, and (3) ribosomal RNA turnover in rat liver is in part regulated through changes in the population of membrane-free ribosomes and subunits, the abundance of which is affected by food deprivation.

Plasma or Serum Free Amino Acids

Since 1906, when Howell reported that the concentration of free amino acids in portal blood increases after a protein meal, the relationship of plasma or serum free amino acids to the amino acid composition of the diet has been studied extensively in various species.

Charkey et al. (1953) and Richardson et al. (1953) were the first to investigate this physiological phenomenon in poultry. Subsequently, a considerable amount of research has been done on this aspect in the past decade. However, there is no attempt to report all of these studies here; therefore, the reviews have been carefully selected within our specific interest.

Dean and Scott (1962), Smith and Scott (1965a,b), and Smith (1966a) have suggested that one could use the plasma amino acid patterns of chicks fed a standard reference diet containing crystalline amino acids to compare with the results from the feeding of an intact protein. It is then proposed to use this information to predict the amino acid in-

adequacies of the protein on the basis of differences in the amino acid patterns for the period from 8 to 14 days of age.

Hill and Olsen (1967) studied plasma free amino acids of chicks fed diets based on soybean meal and showed that a dietary deficiency of methionine resulted in elevated lysine levels in the blood plasma.

Zimmerman and Scott (1967) reported that feeding a non-protein diet lowered the concentration of essential amino acids in the blood plasma below the level noted when chicks were fasted over a comparable period of time (3, 6, 12, or 24 hrs.). With time, several amino acids (methionine, isoleucine, tyrosine and phenylalanine, but, notably, lysine) progressively accumulated in blood plasma under fasting conditions. Threonine was unaffected. Plasma lysine also increased with time when chicks were fed a nonprotein diet, plasma threonine decreased and other amino acids were largely unaffected.

Novacek and Carlson (1968) reported that growth was improved at high levels of soybean and fish meal supplementation to low protein (20%) starter diets for turkeys. There were no associations noted for growth, first limiting amino acids or free amino acids in serum.

Stutz et al. (1971) studied the relationship of dietary cations to arginine-lysine antagonism and free amino acid patterns in chicks. They reported that the supplementation

with either arginine·HCl or sodium and potassium salts of metabolizable acids to a basal purified diet (containing 1.2% arginine, 2.5% lysine, 0.4% potassium and a cation to anion ratio of 1:1) produced marked growth stimulation. The cation supplement stimulated the growth rate to the same extent as did 0.6% arginine, but it decreased the deficiency signs (ataxia and frizzled feathers) typical of arginine deficiency. More significantly, both arginine and potassium acetate supplementation decreased plasma and muscle lysine, threonine and serine while increasing the levels of free arginine. A combination of the supplements gave plasma and muscle amino acid patterns very similar to those observed in chicks fed a practical corn-soybean meal diet. It may be concluded that cation supplementation of this casein-based diet spares the arginine requirement and creates more favorable free amino acid patterns. It is postulated that the rate of arginine catabolism is decreased by the more positive cation balance.

Use of Alpha-aminoisobutyric Acid

Alpha-aminoisobutyric acid (AIB), as well as 1-aminocyclopentane-1-carboxylic acid, is almost totally resistant to metabolic modification in the animal organism. It is excreted exceedingly slowly, and essentially constant levels in the body fluids can easily be obtained during long

experimental intervals. AIB and 1-aminocyclopentane-1-carboxylic acid have been used in amino acid transport or absorption studies by Christensen (1962; 1963) and many other workers.

Smith (1966b) incorporated AIB into the diet of one-week-old chicks to serve as an indicator of amino acid absorption. The levels of AIB and of the metabolizable amino acids in the plasma were subsequently determined. Plasma concentrations of AIB were found to be proportional to dietary levels fed. In general, the indicator revealed that imbalanced diets were not as rapidly absorbed as balanced diets.

Shao and Hill (1969) found that the addition of 0.5% AIB to a casein basal diet (1) improved weight gain and feed efficiency of chicks, (2) caused an accumulation of free arginine and a depression of free ornithine in both plasma and muscle, and (3) greatly depressed kidney arginase. Therefore, they suggested that the possible arginine-sparing effect of AIB supplementation was caused by the inhibition of kidney arginase in the chick fed the casein basal diet, which contained an excess of lysine thus depressing arginine utilization.

Furthermore, they also reported that the AIB supplement had no effect on growth rate and feed efficiency when wheat gluten was used as a basal diet in which the lysine level was adjusted to the National Research Council (1966) requirement.

EXPERIMENTAL PROCEDURE

Management

Three experiments have been conducted with Broad Breasted Bronze male poults. The day-old poults were reared to four weeks of age in wirefloored, electrically heated battery brooders and then transferred to nonheated, intermediate or finisher grower batteries until the end of trials. Both types of batteries were located in a building equipped with a supplemental heating unit, where temperature was maintained at 70°±5° F. Water and ration were available ad libitum for the entire period of the trials.

Experimental Treatments

In Experiment 1, there were 5 ration treatments with all rations isocaloric. Diets 1, 3 and 5 contained 20, 25, and 30% protein, respectively. Diet 2 was the same as 1, which contained 20% protein, but was supplemented with 0.3% arginine, 0.22% methionine and 0.6% lysine. Diet 4 equaled diet 3 (25% protein) with supplementation of 0.11% methionine.

All of the poults were fed a 20% protein diet from hatch to one week of age, at which time they were weighed and randomly assigned to pens; finally, the pen weights were adjusted to as uniform a weight as possible. Poults with extreme body weights were eliminated. There were 12 poults per

pen and 3 pens per treatment. Starting from one week of age the poults were fed the experimental diets shown in Table 1.

Data of body weight and feed consumption were collected at the 3rd and 5th week of age. At 5 weeks poults were decapitated and blood was collected individually from the carotid artery.

In Experiment 2, there were 3 ration treatments with all rations isocaloric and isonitrogenous. Diet 1 contained 27% of protein; diet 2 was as diet 1 but with the supplementation of 0.11% methionine. Diet 3 was as diet 1 but with the supplementation of 0.11% glutamic acid.

During the feeding trial, the poults in treatment 1 were kept on the same ration throughout the entire experimental period. Poults in treatment 2 and 3 were fed the diets for only three weeks (period 1); then the diets were exchanged between these two treatments in the remaining three weeks (period 2). All of the poults were fed a 27% protein diet from hatch to one week of age. The poults were then alloted to each pen as in Experiment 1. There were 15 poults per pen and 4 pens per treatment. Starting from one week of age, the poults were fed the experimental diets shown in Table 2.

At the end of each week of the experimental period, poults were fasted 16 hours and pen weights and feed consumption were recorded; subsequently, one randomly chosen poult per pen was decapitated and blood collected from the carotid

Table 1. Composition of diets used in Experiment 1

Treatment	1	3	5
Protein	20%	25%	30%
Met. Energy (Kcal/lb)	1335	1339	1345
Ingredient	**(%)**	**(%)**	**(%)**
Gr. Yellow Corn	50.0	50.0	50.0
Soybean Meal (48%)	29.0	29.0	29.0
Fish Meal (70%)	3.0	3.0	3.0
Alfalfa Meal (17%)	2.0	2.0	2.0
Defl. Phosphate	2.5	2.5	2.5
Oyster Shell	1.0	1.0	1.0
Trace Mineral Salt[1]	0.5	0.5	0.5
Vitamin Mix[2]	0.5	0.5	0.5
Dextrose	11.5	5.7	0
Soy Protein	0	5.8	11.5

Treatment 2 = Treatment 1 + 0.3% Arginine + 0.22% Methionine + 0.6% Lysine

Treatment 4 = Treatment 3 + 0.11% Methionine

[1]Trace Mineralized Salt mix provided (per kilogram of diet): NaCl 2 gms, Zn 34 mg, Fe 25 mg, I_2 0.5 mg, Co 0.25 mg, Cu 4 mg.

[2]Vitamin mix provided (per kilogram of diet): A-10,000 IU, D_3-2,200 ICU, E-10 IU, Menadione Sodium Bisulfite 2.0 mg, B_{12} 10 mcg, B_2 5.3 mg, Pantothenic acid 10 mg, Niacin 60 mg, Choline 450 mg, Folacin 0.55 mg, Santoquin 0.0125%.

Table 2. Composition of diets used in Experiments 2 and 3

Experiment	2	3	3
Treatment	1	1	4
Protein	27%	25%	30%
Met. Energy (Kcal/lb)	1337	1332	1338
Ingredient	(%)	(%)	(%)
Gr. Yellow Corn	50.0	50.0	50.0
Soybean Meal (48%)	29.0	29.0	29.0
Fish Meal (70%)	3.0	3.0	3.0
Alfalfa Meal (17%)	2.0	2.0	2.0
Defl. Phosphate	2.5	2.5	2.5
Oyster Shell	1.0	1.0	1.0
Trace Mineral Salt[1]	0.5	0.5	0.5
Vitamin Mix[1]	0.5	0.5	0.5
Soy Protein	5.8	4.0	11.1
Dextrose	5.6	7.1	0
AIB[2]	0.1	0.4	0.4

[1]Trace Mineralized Salt mixes and Vitamin mixes were the same as Experiment 1.

[2]Alpha-aminoisobutyric acid.

artery. The entire pancreas from this poult was taken after blood collection.

In Experiment 3, there were 4 ration treatments with all rations isocaloric. Diet 1 contained 25% of protein; diet 2 was the same as diet 1, but with the supplementation of 0.11% methionine; diet 3 was as diet 1, but with the supplementation of 0.11% glutamic acid. Diet 4 contained 30% of protein.

Poults from treatments 1 and 4 were fed the diets throughout the entire experimental period (6 weeks). However, diets of poults in treatments 2 and 3 were exchanged at the half way point of the experimental period. All of the poults were fed a diet with 25% of protein from hatch to one week of age; at that time, poults were assigned to each pen as in Experiment 1. There were 11 poults per pen, and 4 pens per treatment. Starting from one week of age, the poults were fed the experimental diets shown in Table 2.

The method followed to collect data of pen weights, feed consumption, blood and pancreas were same as in Experiment 2. In addition, at the 4th and 7th week of age a liver sample of the one sacrificed poult from each pen was saved for chemical analysis.

The comparison of National Research Council (1966) requirement and calculated essential amino acids of Experiments 2 and 3 are presented in Table 3.

Table 3. The comparison of National Research Council requirement and calculated essential amino acids of Experiments 2 and 3

Amino Acid	N.R.C. Req. (1966)	Expt. 2 (27% Pro.)	Expt. 3 (25% Pro.)	Expt. 3 (30% Pro.)
	(%)	(%)	(%)	(%)
Arginine	1.60	1.72	1.61	2.08
Histidine	?	0.62	0.58	0.75
Lysine	1.50	1.55	1.46	1.86
Tryptophan	0.26	0.31	0.29	0.35
Phenylalanine	?(0.98)	1.32	1.24	1.60
Tyrosine	?(0.84)	0.92	0.88	1.13
Methionine	0.52	0.44	0.42	0.51
Cystine	0.35	0.38	0.36	0.45
Threonine	?(0.98)	1.01	0.96	1.19
Leucine	?(1.96)	2.20	2.07	2.62
Isoleucine	0.84	1.32	1.25	1.56
Valine	?(1.19)	1.32	1.25	1.57
Glycine	1.00	1.26	1.20	1.46
AIB[1] Added		0.10	0.40	0.40

[1]Alpha-aminoisobutyric acid.

Chemical Determinations

Pancreas study

After collection, pancreases were weighed immediately and stored in small plastic bags in a walk-in deep freezer (temperature -15°C) until chemical analysis at the end of experiment. The frozen pancreases were homogenized with 1:250 ml of ice-cold, distilled water; then the homogenized tissue was filtrated in the walk-in cooler (temperature 4°C). Aliquots of filtrate were collected for further chemical determination. The Biuret method described by Layne (1957) was used for soluble protein determination. Proteolytic enzyme activity was measured according to Anson (1938) and Lepkovsky et al. (1964).

Liver DNA, RNA and protein study

Livers were weighed immediately after collection and stored in small plastic bags in the walk-in deep freezer. Munro's (1969) method was used for liver DNA and RNA measurement. Protein was determined by Micro-Kjeldahl of A.O.A.C. (1965).

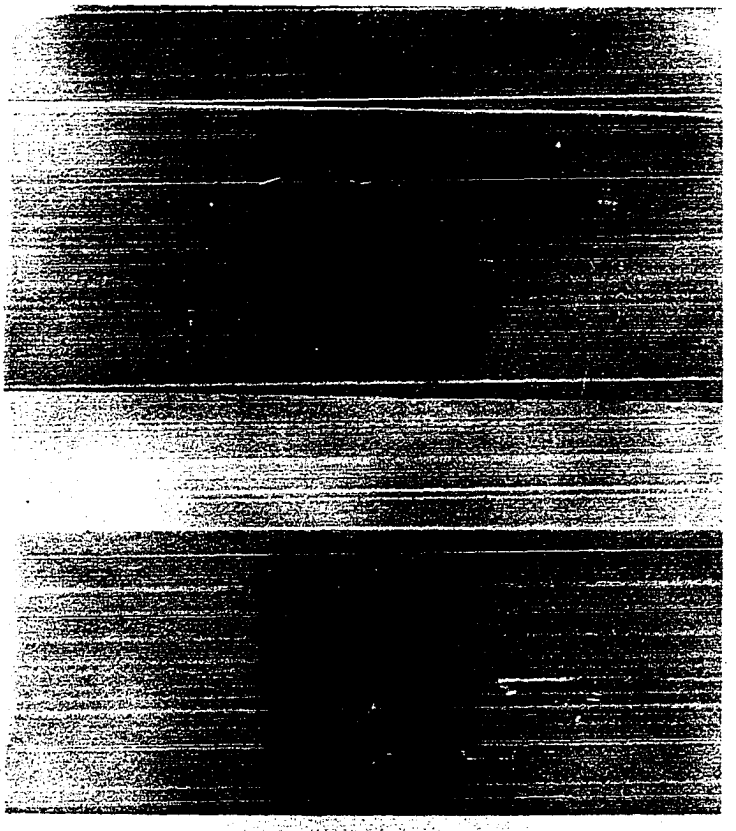
Serum study

Blood samples were collected from the carotid artery of the poults into test tubes and were allowed to coagulate for 8 to 10 hrs at room temperature. Serum samples were frozen and stored in vials until the chemical determinations could

be made. The T/C Refractometer of American Optical Instrument Company was used for serum protein determination. Serum albumin and globulin (Fig. 1) were separated by cellulose polyacetate strips which were processed by using equipment and technique referred to as the Gelman Electrophoresis System (Anonymous, 1966). Serum free amino acids (Fig. 2) were measured by Technicon Amino Acid Autoanalyzer (Stein and Moore, 1954; Moore et al. 1958).

Figure 1. Serum albumin and globulin were separated by electrophoresis on cellulose polyacetate strips in the upper picture. The heaviest band indicates albumin portion.

The duplicate strips were scanned by densitometer in the lower picture. Albumin bands had highest peaks.



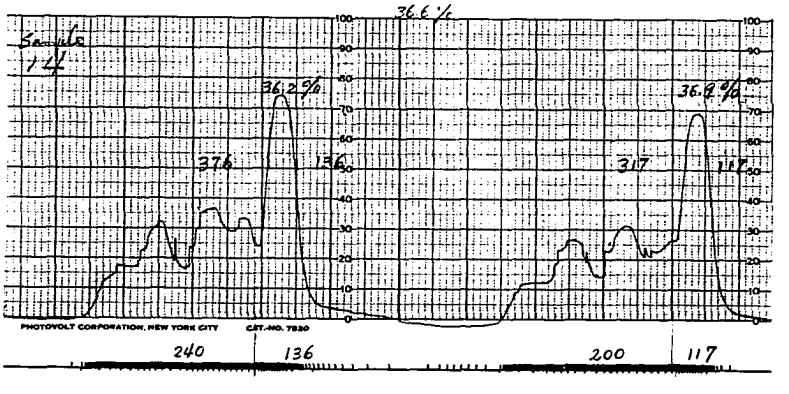

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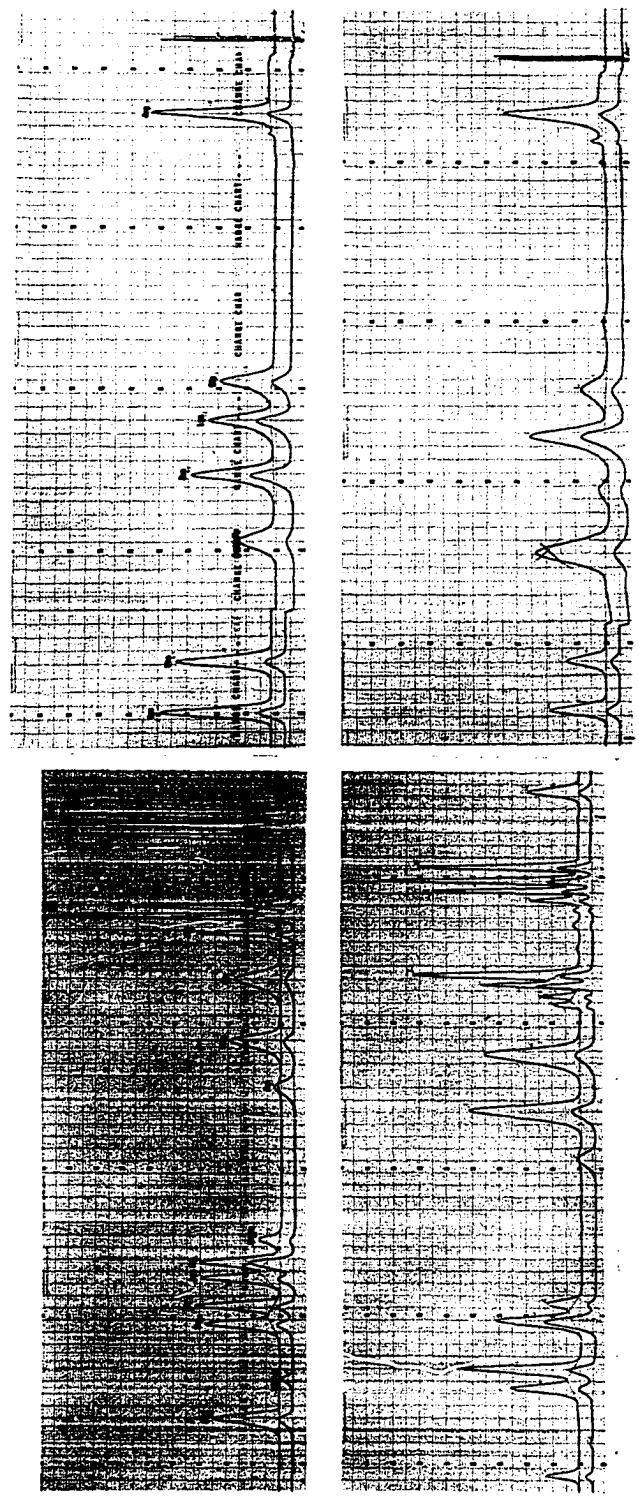
Figure 2. Chromatogram of serum free amino acids which were measured by Technicon Amino Acid Autoanalyzer. The upper picture indicates a standard and the lower picture indicates a sample.



Statistical Analysis

A completely randomized design was used in Experiment 1. A mixture of completely randomized and cross-over design was applied in Experiments 2 and 3 (Table 4). The methods of statistical analysis were as described by Cochran and Cox (1968), Steel and Torrie (1960) and Snedecor and Cochran (1967). The Statistical Laboratory of Iowa State University assisted in computer analysis of variance and orthogonal comparisons.

Table 4. The statistical design of Experiments 2[1] and 3

Treatment	Period 1 (week of age)			Period 2 (week of age)		
	2	3	4	5	6	7
1	Low Protein			Low Protein		
2	Low Protein+Methionine			Low Protein+Glutamic Acid		
3	Low Protein+Glutamic Acid			Low Protein+Methionine		
4	High Protein			High Protein		

[1]Experiment 2 had no treatment 4.

RESULTS

Experiment 1

Average body gains at the 3rd and 5th week of age are shown in Table 5. Treatment 2 supplemented with the limiting amino acids arginine, methionine and lysine, and treatment 4 supplemented with methionine, had significantly greater average weight gains at both the 3rd and 5th week of age.

Among treatments 1, 3 and 5, which were the treatments without supplementing amino acids, there was a linear increase in gain with protein level increasing in the diet. The only statistically significant comparison was treatment 1 versus the other treatments at either the 3rd or 5th week of age.

Feed efficiency data are presented in Table 5. Poults in treatments 2 and 4 had better feed conversions than those in treatments 1 and 3. Excluding the supplemented amino acid treatments, feed efficiency was improved when dietary protein level was increased. The statistically significant differences are also shown in Table 5.

Serum protein and albumin are illustrated in Table 6. Amino acid supplementation of the diets resulted in increased serum protein and albumin. However, among treatments 1, 3 and 5, serum protein showed constant values as dietary protein was

Table 5. Average body weight gain and feed efficiency in Experiment 1

Treatment	Gain(gm)/Poult[1] 3wks	5wks	Feed/Gain[1] 3wks	5wks
1 20% Protein	206[c]	501[c]	1.71[c]	2.18[c]
2 1+Arg+Meth+Lys	290[a]	656[a]	1.51[ab]	1.95[ab]
3 25% Protein	255[b]	594[b]	1.58[b]	2.00[b]
4 3+Meth	283[a]	664[a]	1.47[a]	1.88[ab]
5 30% Protein	259[b]	644[ab]	1.50[ab]	1.83[a]

[1]Means with the same superscript are not significantly different based on Duncan's Multiple-Range Test. (Probability = 0.05 or less)

Table 6. Total serum protein and albumin in Experiment 1

Treatment	1	2	3	4	5
Protein (gm/100ml)	3.2	3.5	3.1	3.7	3.0
Albumin (%)	48.7	57.7	51.5	58.8	55.4

increased. The albumin tended to increase as dietary protein was increased, but there were no statistically significant differences observed.

Experiment 2

Mean weight gains are shown in Table 7. Poults from treatment 2, with methionine supplement, had greater average weight gains than poults from treatment 1 and 3 in period 1. In period 2, the poults from treatment 3, with methionine supplementation, increased their rate of gain significantly, while poults from treatment 2 were depressed in weight gains.

Feed efficiency data are presented in Table 8. In period 1, treatment 2 produced the best feed efficiency among the three treatments. In period 2, poults from treatment 3 improved their feed efficiency significantly, while poults from treatment 2 showed poor feed efficiency.

There were no significant differences observed among treatment groups in pancreas size (Table 9). However, poults from treatment 2 in period 1 and treatment 3 in period 2, tended to have more pancreas soluble protein than the other treatment groups (Table 10). Pancreas size and soluble protein increased linearly with age.

Poults from treatment 3 in period 1, and treatment 2 in period 2 (treatments with added glutamic acid) had a high pancreas proteolytic enzyme specific activity at the begin-

Table 7. Average body weight gain (gm) per poult per week in Experiment 2

Treatment[1]	Period 1 (wks) 2	3	4		Period 2 (wks) 5	6	7
1 (L.P.)	64.9	103.1	153.5	(L.P.)	214.7	287.8	361.4
2 (L.P.+M)	74.9	123.5	173.4	(L.P.+G)	225.6	278.3	341.9
3 (L.P.+G)	58.1	101.7	147.6	(L.P.+M)	232.9	299.6	365.9

[1]L.P. means low protein (27%); L.P.+M means low protein with supplementary methionine; L.P.+G means low protein with added glutamic acid. In this table and tables through 17.

Table 8. Feed efficiency (feed intake/gain/week) in Experiment 2

Treatment[1]	Period 1 (wks) 2	3	4		Period 2 (wks) 5	6	7
1 (L.P.)	1.83	2.00	1.94	(L.P.)	2.13	2.29	2.39
2 (L.P.+M)	1.66	1.76	1.89	(L.P.+G)	2.13	2.40	2.53
3 (L.P.+G)	1.91	1.92	2.08	(L.P.+M)	1.96	2.17	2.33

Table 9. Average pancreas weight per poult (gm) in Experiment 2

Treatment[1]	Period 1 (wks) 2	3	4		Period 2 (wks) 5	6	7
1 (L.P.)	0.67	1.25	1.73	(L.P.)	2.67	3.81	4.17
2 (L.P.+M)	0.83	1.36	1.97	(L.P.+G)	2.92	3.93	5.14
3 (L.P.+G)	0.74	1.40	1.74	(L.P.+M)	2.90	3.68	5.26

Table 10. Total soluble protein of pancreas (mg) in Experiment 2

Treatment[1]	Period 1 (wks) 2	3	4		Period 2 (wks) 5	6	7
1 (L.P.)	108	163	364	(L.P.)	602	845	926
2 (L.P.+M)	163	270	410	(L.P.+G)	590	783	1163
3 (L.P.+G)	110	214	354	(L.P.+M)	623	831	1345

Table 11. Proteolytic specific activity[2] of pancreas in Experiment 2

Treatment[1]	Period 1 (wks) 2	3	4		Period 2 (wks) 5	6	7
1 (L.P.)	0.37	0.30	0.37	(L.P.)	0.18	0.19	0.21
2 (L.P.+M)	0.26	0.28	0.22	(L.P.+G)	0.24	0.28	0.20
3 (L.P.+G)	0.42	0.37	0.24	(L.P.+M)	0.18	0.19	0.14

[2] S.A. = $\frac{\text{Absorbance}}{\text{Protein (mg)}}$ --- Lepkovsky (1964) & Anson (1938) --- Biuret

ning of the trials which decreased sharply at the end (Table 11). In addition, treatment 1 caused a similar trend in which the specific activities were high in period 1 and decreased significantly in period 2.

No differences were observed in serum protein (Table 12) or serum albumin (Table 13) among poults from the various treatments. However, the percentage of serum albumin increased significantly with age.

There were no statistically significant difference in serum free amino acids among treatments at any age from 2 to 7 weeks of age (Table 14 and 15). However, lysine, glycine, threonine and glutamic acid had highly significantly greater concentrations in period 1 than 2. Since these four amino acids were a large portion of the total serum amino acids, the total essential and non-essential free amino acids of serum showed a declining trend with age.

When serum free amino acids were measured by the ratio to alpha-aminoisobutyric acid (AIB) (Table 16 and 17), only threonine had an observed significant interaction effect between dietary treatment and age on treatment 1 versus 3, in period 2.

Table 12. Total serum protein (gm/100ml) in Experiment 2

Treatment[1]	Period 1 (wks) 2	3	4		Period 2 (wks) 5	6	7
1 (L.P.)	3.9	4.1	3.5	(L.P.)	3.5	4.2	3.8
2 (L.P.+M)	4.0	3.7	3.5	(L.P.+G)	3.4	3.9	4.1
3 (L.P.+G)	4.5	3.7	3.4	(L.P.+M)	4.2	3.6	4.2

Table 13. Serum albumin percentage[2] in Experiment 2

Treatment[1]	Period 1 (wks) 2	3	4		Period 2 (wks) 5	6	7
1 (L.P.)	41.2	39.2	41.7	(L.P.)	43.5	44.9	44.9
2 (L.P.+M)	41.3	39.6	41.6	(L.P.+G)	44.4	43.1	42.7
3 (L.P.+G)	43.1	39.0	37.2	(L.P.+M)	38.6	47.0	47.9

[2] $\text{Albumin\%} = \frac{\text{Albumin}}{\text{Albumin + Globulin}} \times 100\%$

Table 14. Means of serum essential free amino acids in Experiment 2

Treatment[1]	Age wks	Serum essential free amino acids (mg/100ml)												
		Arg	Lys	His	Meth	Cys	Gly	Phe	Tyr	Leu	Iso	Thr	Val	Total
1 (L.P.)	2	9.44	14.66	1.30	1.19	2.21	8.27	2.71	2.86	4.33	3.54	6.19	5.90	62.60
	3	7.94	16.86	2.74	1.15	3.43	6.87	3.01	3.73	4.75	3.13	5.25	4.86	63.72
	4	7.48	10.64	2.11	1.01	1.44	5.06	2.34	2.95	3.62	2.36	4.08	4.44	47.53
(L.P.)	5	8.17	11.05	2.61	1.23	2.79	5.55	2.91	3.43	3.85	2.56	4.88	4.25	53.28
	6	7.70	7.98	0.69	1.28	3.52	5.92	3.10	3.47	4.36	2.66	5.73	6.04	52.45
	7	8.23	9.42	1.51	1.66	3.52	5.73	3.16	4.24	6.01	3.46	4.99	6.42	58.35
2 (L.P.+M)	2	10.65	18.61	1.37	1.50	1.70	8.42	2.82	3.33	5.86	3.76	5.36	5.52	68.90
	3	8.84	15.94	1.61	1.26	2.49	5.71	3.10	3.76	5.42	2.94	5.29	5.02	61.38
	4	8.24	14.53	1.00	1.26	2.51	5.29	2.88	4.08	5.01	2.31	4.49	3.78	55.38
(L.P.+G)	5	7.46	10.20	2.42	1.52	3.03	5.33	2.89	3.62	4.69	3.21	4.32	5.24	53.93
	6	7.85	10.27	1.78	1.37	2.94	5.04	2.64	3.30	4.10	2.80	3.13	4.47	49.69
	7	9.08	8.49	0.91	1.59	4.06	3.99	3.04	4.08	4.89	3.38	3.60	5.54	52.65
3 (L.P.+G)	2	9.26	23.62	1.42	1.01	2.46	7.46	2.82	3.36	5.29	3.57	6.42	4.92	71.61
	3	7.74	11.34	2.55	1.22	2.78	5.90	2.67	3.14	3.88	2.77	5.61	5.74	55.34
	4	5.51	9.96	1.35	1.02	1.47	5.45	2.52	3.74	3.77	2.72	4.36	4.37	46.24
(L.P.+M)	5	8.49	8.40	1.36	1.30	2.33	4.88	3.21	3.57	5.29	2.69	4.09	4.72	50.33
	6	7.40	7.77	0.72	1.34	3.51	5.59	2.75	3.27	4.28	2.42	3.64	4.10	46.79
	7	10.33	9.41	2.17	1.72	4.15	4.78	3.43	3.77	5.66	3.40	3.04	6.08	57.94

Table 15. Means of serum non-essential free amino acids in Experiment 2

Treatment1	Age wks	Serum non-essential free amino acids (mg/100ml)								
		Ala	Asp	HO-P	Glu	Pro	Orn	Ser	Total	AIB
1 (L.P.)	2	5.95	1.33	0.53	7.68	4.43	0.97	5.60	26.49	1.56
	3	7.15	2.41	0.87	7.80	3.87	1.00	6.31	29.41	1.12
	4	6.26	1.53	0.54	4.83	3.13	0.79	4.72	21.80	0.90
(L.P.)	5	5.74	1.56	0.72	5.55	3.45	0.91	4.80	22.73	0.92
	6	6.29	1.68	0.49	6.86	3.99	0.87	5.86	26.04	0.68
	7	6.44	1.42	1.21	6.20	3.64	1.30	4.64	24.85	0.77
2 (L.P.+M)	2	5.47	1.23	0.41	8.61	3.86	1.07	5.11	25.76	1.83
	3	5.92	1.45	0.48	6.63	3.45	1.38	4.40	23.71	0.83
	4	6.56	1.48	0.91	5.95	3.55	0.95	5.58	24.98	0.88
(L.P.+G)	5	5.31	1.30	0.42	5.17	3.01	0.82	4.97	21.00	0.97
	6	5.51	1.21	0.57	4.37	2.96	0.67	5.46	20.75	0.58
	7	6.60	1.36	0.96	6.51	3.03	0.89	4.09	23.44	0.80
3 (L.P.+G)	2	4.72	1.40	0.57	8.18	3.65	1.24	4.68	24.44	1.27
	3	7.69	1.69	0.55	8.56	4.04	2.17	5.78	30.48	0.99
	4	6.95	1.29	0.54	5.17	3.53	0.91	5.28	23.67	0.91
(L.P.+M)	5	4.90	1.18	0.31	4.76	3.32	1.09	3.48	19.04	0.71
	6	5.87	1.31	0.42	3.72	3.25	0.59	5.58	20.74	1.08
	7	6.14	1.19	0.99	6.24	3.39	0.98	3.72	22.65	0.67

Table 16. The ratio of serum essential free amino acids to AIB in Experiment 2

Treatment[1]	Age wks	Serum essential free amino acids / AIB												
		Arg	Lys	His	Meth	Cys	Gly	Phe	Tyr	Leu	Iso	Thr	Val	Total
1 (L.P.)	2	6.05	9.40	0.83	0.76	1.42	5.30	1.74	1.83	3.78	2.27	3.97	3.78	40.13
	3	7.09	15.05	9.04	1.03	3.06	6.13	2.69	3.33	4.24	2.79	4.69	4.34	56.89
	4	8.31	11.82	2.34	1.12	1.60	5.62	2.60	3.28	4.02	2.62	4.53	4.93	52.79
(L.P.)	5	8.88	12.01	2.84	1.34	3.03	6.03	3.16	3.73	4.18	2.78	5.30	4.62	57.90
	6	11.32	11.74	1.01	1.88	5.18	8.71	4.56	5.10	6.41	3.91	8.43	8.88	77.13
	7	10.69	12.23	1.96	2.16	4.57	7.44	4.10	5.51	7.81	4.49	6.48	8.34	75.78
2 (L.P.+M)	2	5.82	10.17	0.75	0.82	0.93	4.60	1.54	1.82	3.20	2.05	2.93	3.02	37.65
	3	10.62	19.20	1.94	1.52	3.00	6.88	3.73	4.53	6.53	3.54	6.37	6.05	73.94
	4	9.36	16.51	1.14	1.43	2.86	6.01	3.27	4.64	5.69	2.63	5.10	4.30	62.94
(L.P.+G)	5	7.69	10.52	2.49	1.57	3.12	5.49	2.98	3.73	4.84	3.31	4.45	5.40	55.59
	6	13.53	17.71	3.07	2.36	5.07	8.69	4.55	5.69	7.07	4.83	5.40	7.71	85.68
	7	11.35	10.61	1.14	1.99	5.08	4.99	3.80	5.10	6.11	4.23	4.50	6.93	65.83
3 (L.P.+G)	2	7.29	18.60	1.12	0.80	1.94	5.87	2.22	2.65	4.17	2.81	5.06	3.87	56.40
	3	7.82	11.45	2.58	1.23	2.81	5.96	2.70	3.17	3.92	2.80	5.67	5.80	55.91
	4	6.05	10.95	1.48	1.12	1.62	5.99	2.77	4.11	4.14	2.99	4.79	4.80	50.81
(L.P.+M)	5	11.96	11.83	1.92	1.83	3.28	6.87	4.52	5.03	7.45	3.79	5.76	6.65	70.89
	6	6.85	7.19	0.67	1.24	3.25	5.18	2.55	3.03	3.96	2.24	3.37	3.80	43.33
	7	15.42	14.04	3.24	2.57	6.19	7.13	5.12	5.63	8.45	5.07	4.54	9.07	86.47

Table 17. Ratio of serum non-essential free amino acids to AIB in Experiment 2

Treatment[1]	Age wks	Serum non-essential free amino acids/AIB							
		Ala	Asp	HO-P	Glu	Pro	Orn	Ser	Total
1 (L.P.)	2	3.81	0.85	0.34	4.92	2.84	0.62	3.59	16.97
	3	6.38	2.15	0.78	7.00	3.46	0.89	5.63	26.29
	4	6.96	1.70	0.60	5.37	3.48	0.88	5.24	24.23
(L.P.)	5	6.24	1.70	0.78	6.03	3.75	0.99	5.22	24.71
	6	9.25	2.47	0.72	10.09	5.87	1.28	8.62	38.30
	7	8.36	1.84	1.57	8.05	4.73	1.69	6.03	32.27
2 (L.P.+M)	2	2.99	0.67	0.22	4.70	2.11	0.58	2.79	14.06
	3	7.13	1.75	0.58	7.99	4.16	1.66	5.30	28.57
	4	7.45	1.68	1.03	6.76	4.03	1.08	6.34	28.37
(L.P.+G)	5	5.47	1.34	0.43	5.33	3.10	0.85	5.12	21.64
	6	9.50	2.09	0.98	7.53	5.10	0.16	9.41	35.77
	7	8.25	1.70	1.20	8.14	3.79	1.11	5.11	29.30
3 (L.P.+G)	2	3.72	1.10	0.45	6.44	2.87	0.98	3.69	19.25
	3	7.77	1.71	0.56	8.65	4.08	2.19	5.84	30.80
	4	7.64	1.42	0.59	5.68	3.88	1.00	5.80	26.01
(L.P.+M)	5	6.90	1.66	0.44	6.70	4.68	1.54	4.90	26.82
	6	5.44	1.21	0.39	3.44	3.01	0.55	5.17	19.21
	7	9.16	1.78	1.48	9.31	5.06	1.46	5.55	33.80

Experiment 3

Mean body weight gains per poult are shown in Table 18. Poults from treatment 2 (methionine supplementation) and treatment 4 (high protein) had significantly greater gains than those from treatments 1 and 3 in period 1. However, when rations were exchanged between treatments 2 and 3 in period 2, the gain per poult from treatments 3 and 4 were significantly greater than from treatments 1 and 2.

Feed efficiency data are presented in Table 19. Poults from treatments 2 and 4 had better feed efficiency than those from treatments 1 and 3 in period 1. But when rations 2 and 3 were exchanged in period 2, treatments 3 and 4 resulted in better feed conversion than did treatment 1 and 2. However, the differences were statistically significant only between treatments 2 and 4 in period 2.

Data of pancreas size are presented in Table 20. Treatment 4 caused significantly greater pancreas weights than the rest of the treatments. Poults fed a diet with methionine supplementation tended to have slightly heavier pancreases in both period 1 and 2. As expected, pancreas weight increased linearly with age. Total pancreas soluble protein is shown in Table 21. In the entire period of the trial, poults from treatments 3 and 4 had significantly larger amount of pancreas protein than those from treatment 1 and 2, respectively. In all the treatments, the amount of protein increased

Table 18. Average body weight gain (gm) per poult per week in Experiment 3

Treatment[1]	Period 1 (wks) 2	3	4		Period 2 (wks) 5	6	7
1 (L.P.)	67.0	101.0	143.0	(L.P.)	211.1	264.5	301.9
2 (L.P.+M)	82.9	122.6	161.2	(L.P.+G)	219.1	258.8	312.5
3 (L.P.+G)	69.2	102.2	140.7	(L.P.+M)	242.9	269.0	337.7
4 (H.P.)	72.6	114.6	162.3	(H.P.)	229.3	290.6	328.4

[1]L.P. means low protein (25%); L.P.+M means low protein with supplementary methionine; L.P.+G means low protein with added glutamic acid; H.P. means high protein (30%). In this table and tables through 29.

Table 19. Feed efficiency (feed intake/gain/week) in Experiment 3

Treatment[1]	Period 1 (wks) 2	3	4		Period 2 (wks) 5	6	7
1 (L.P.)	1.85	2.01	2.05	(L.P.)	2.15	2.31	2.47
2 (L.P.+M)	1.68	1.81	1.96	(L.P.+G)	2.27	2.46	2.51
3 (L.P.+G)	1.90	1.96	2.17	(L.P.+M)	2.03	2.31	2.39
4 (H.P.)	1.80	1.87	1.92	(H.P.)	2.24	2.26	2.30

Table 20. Average pancreas weight per poult (gm) in Experiment 3

Treatment[1]	Period 1 (wks) 2	3	4		Period 2 (wks) 5	6	7
1 (L.P.)	0.82	1.10	2.00	(L.P.)	2.40	2.70	3.34
2 (L.P.+M)	0.78	1.21	2.00	(L.P.+G)	2.44	2.84	3.13
3 (L.P.+G)	0.85	1.20	1.80	(L.P.+M)	2.74	3.10	3.51
4 (H.P.)	0.82	1.40	2.10	(H.P.)	3.01	3.51	3.84

Table 21. Total soluble protein of pancreas (mg) in Experiment 3

Treatment[1]	Period 1 (wks) 2	3	4		Period 2 (wks) 5	6	7
1 (L.P.)	228	195	380	(L.P.)	544	628	728
2 (L.P.+M)	205	310	356	(L.P.+G)	566	594	830
3 (L.P.+G)	209	276	469	(L.P.+M)	703	765	994
4 (H.P.)	298	280	489	(H.P.)	791	903	1156

Table 22. Proteolytic specific activity[2] of pancreas in Experiment 3

Treatment[1]	Period 1 (wks) 2	3	4		Period 2 (wks) 5	6	7
1 (L.P.)	0.40	0.41	0.28	(L.P.)	0.24	0.16	0.33
2 (L.P.+M)	0.23	0.24	0.09	(L.P.+G)	0.22	0.27	0.13
3 (L.P.+G)	0.38	0.28	0.15	(L.P.+M)	0.12	0.20	0.12
4 (H.P.)	0.15	0.31	0.16	(H.P.)	0.15	0.17	0.13

[2]See footnotes Table 11.

linearly with age. Poults from treatment 1 had significantly greater proteolytic specific activities than those from the other treatments during the entire experimental period (Table 22). Treatment with glutamic acid supplementation tended to cause greater activities than the treatment with methionine supplementation.

The liver DNA density, RNA:DNA ratio, and protein percentage are presented in Table 23. Comparing 4 with 7 weeks of age, DNA density tended to decrease. In general, a high protein or methionine supplemented diet resulted in a greater density of DNA in the liver than did the low protein diet at both 4 and 7 weeks of age. Furthermore, glutamic acid supplementation caused the highest DNA density among the various treatments at 4 weeks of age. However, differences in RNA: DNA ratio were not statistically significant. Poults from treatments 3 and 4 had siginificantly more liver protein than from treatments 1 and 2 at the 7th week of age. The treatments caused no difference at the 4th week of age.

No significant differences were observed in total serum protein (Table 24) or serum albumin percentage (Table 25). However, the high protein treatment poults had slightly more serum albumin than poults fed low protein diets in the entire experimental period. In addition, the serum albumin increased significantly with age.

As in Experiment 2, there were no statistically

Table 23. Average liver DNA density, RNA:DNA ratio and protein in Experiment 3

Treatment[1]	4 weeks of age				7 weeks of age		
	DNA (mg/gm)	RNA/DNA	Pro. (%)		DNA (mg/gm)	RNA/DNA	Pro. (%)
1 (L.P.)	1.66	2.68	19.3	(L.P.)	1.48	3.44	19.1
2 (L.P.+M)	1.63	3.02	20.8	(L.P.+G)	1.51	3.64	19.5
3 (L.P.+G)	1.86	3.08	20.8	(L.P.+M)	1.51	3.36	21.3*
4 (H.P.)	1.71	2.98	20.3	(H.P.)	1.67	2.52	21.7*

*Probability = 0.05 or less.

Table 24. Total serum protein (gm/100ml) in Experiment 3

Treatment[1]	Period 1 (wks)				Period 2 (wks)		
	2	3	4		5	6	7
1 (L.P.)	3.3	3.5	3.5	(L.P.)	3.9	3.6	3.2
2 (L.P.+M)	3.9	3.3	3.3	(L.P.+G)	3.5	2.6	3.6
3 (L.P.+G)	3.8	3.5	3.5	(L.P.+M)	3.4	3.2	3.9
4 (H.P.)	3.2	3.2	3.1	(H.P.)	3.3	3.0	3.2

Table 25. Serum albumin percentage[2] in Experiment 3

Treatment[1]	Period 1 (wks)				Period 2 (wks)		
	2	3	4		5	6	7
1 (L.P.)	45.7	46.5	49.0	(L.P.)	45.6	52.0	56.2
2 (L.P.+M)	46.6	53.2	49.9	(L.P.+G)	49.2	57.6	56.2
3 (L.P.+G)	44.2	46.0	48.8	(L.P.+M)	49.9	53.9	53.8
4 (H.P.)	47.2	47.3	55.0	(H.P.)	46.2	58.0	57.5

[2]See footnotes Table 13.

significant differences observed among treatments in serum free amino acids from 2 to 7 weeks of age (Table 26 and 27). However, lysine, cystine, glycine, phenylalanine, leucine, isoleucine, threonine and ornithine had a significantly greater concentration in period 1 than 2. Therefore, the total essential free amino acids of serum showed a declining trend with age. But the total non-essential amino acids showed no such trend.

When serum free amino acids were measured by the ratio to AIB (Table 28 and 29), arginine, lysine, methionine, glycine, phenylalanine, leucine, isoleucine, threonine, alanine, aspartic acid and serine had a significant interaction effect between dietary treatment and age on treatment 2 versus 4.

Table 26. Means of serum essential free amino acids in Experiment 3

Treatment[1]	Age wks	Serum essential free amino acids (mg/100ml)												
		Arg	Lys	His	Meth	Cys	Gly	Phe	Tyr	Leu	Iso	Thr	Val	Total
1 (L.P.)	2	9.80	17.86	1.72	1.45	4.80	6.51	2.84	3.64	5.00	3.29	5.31	4.06	66.28
	3	8.38	15.10	3.15	1.32	4.46	6.08	2.82	3.27	4.25	2.92	4.30	3.87	59.92
	4	7.66	8.22	1.56	1.07	3.97	5.35	2.28	3.38	4.06	1.85	2.77	2.68	44.85
(L.P.)	5	9.37	8.80	0.76	1.00	3.93	6.05	2.37	3.69	3.78	2.07	3.43	3.28	48.53
	6	7.94	8.07	2.19	1.17	2.74	4.12	2.14	4.69	3.30	2.30	3.28	3.11	45.05
	7	7.70	6.25	1.02	0.99	1.87	5.02	2.21	3.35	3.75	2.11	3.04	3.47	40.78
2 (L.P.+M)	2	10.05	18.29	1.20	1.34	5.01	5.98	2.97	3.41	4.87	3.05	4.74	4.24	65.15
	3	9.08	14.44	1.09	1.16	3.97	6.09	2.90	4.01	4.16	2.19	4.85	3.31	57.25
	4	9.29	9.82	2.03	1.18	4.90	5.16	2.92	3.32	5.57	2.45	2.52	4.36	53.52
(L.P.+G)	5	9.30	7.63	1.22	1.19	4.11	6.86	2.57	4.28	4.67	2.24	4.42	4.42	52.91
	6	7.33	8.02	0.89	1.12	2.01	3.96	1.84	2.65	3.10	2.08	3.58	2.45	39.03
	7	7.81	7.18	1.62	1.08	2.84	4.84	2.29	3.36	3.53	2.17	3.40	3.22	43.34
3 (L.P.+G)	2	9.90	14.51	1.55	0.95	4.27	5.63	2.89	3.56	3.74	2.41	3.64	3.01	56.06
	3	7.53	16.61	1.95	1.13	3.85	6.68	2.76	3.61	4.38	2.69	5.86	3.99	61.04
	4	7.72	8.02	0.84	1.07	4.08	5.53	2.62	2.96	4.02	2.12	4.28	3.81	47.07
(L.P.+M)	5	9.28	6.07	0.98	1.22	3.49	5.70	1.93	5.07	4.03	1.95	4.23	4.19	48.14
	6	8.67	7.08	0.92	1.33	3.49	4.29	2.28	3.22	3.93	1.92	3.44	6.32	46.89
	7	9.48	6.99	0.86	1.16	2.72	4.29	2.25	3.22	4.02	2.20	3.35	3.83	44.37
4 (H.P.)	2	11.87	21.99	1.20	1.19	5.09	5.02	3.09	3.23	5.75	3.06	5.36	3.88	70.73
	3	8.54	14.43	1.31	1.43	4.56	5.24	3.11	3.21	5.85	3.44	4.59	4.94	60.65
	4	6.89	8.37	0.89	0.93	3.98	4.17	2.43	3.39	4.34	2.17	2.95	3.48	43.99
(H.P.)	5	8.96	7.92	0.96	1.20	5.83	4.95	2.70	4.56	4.67	2.44	3.82	5.16	53.17
	6	6.29	5.88	0.86	1.18	3.13	4.12	2.13	3.25	2.86	2.09	3.28	3.69	38.76
	7	6.59	6.44	1.19	1.08	2.61	4.42	2.09	2.99	3.09	2.00	4.44	3.58	40.52

Table 27. Means of serum non-essential free amino acids in Experiment 3

Treatment[1]	Age wks	Serum non-essential free amino acids (mg/100ml)								
		Ala	Asp	HO-P	Glu	Pro	Orn	Ser	Total	AIB
1 (L.P.)	2	5.96	1.44	0.59	8.39	3.39	1.08	4.71	25.56	3.86
	3	6.17	1.52	0.92	7.47	3.46	1.17	4.58	25.29	2.52
	4	5.04	1.13	0.77	6.27	2.60	0.87	4.74	21.42	2.46
(L.P.)	5	7.45	1.42	0.66	8.38	2.94	0.58	5.03	26.46	2.24
	6	5.71	1.03	0.53	5.36	2.19	0.55	4.30	19.67	1.58
	7	7.48	1.33	1.29	6.03	2.96	0.55	5.45	25.09	1.90
2 (L.P.+M)	2	6.66	1.40	0.74	7.89	3.65	1.59	3.98	25.91	4.35
	3	8.19	1.60	0.89	7.84	3.35	1.05	5.88	28.80	2.97
	4	6.79	1.24	0.98	6.79	3.91	1.12	4.96	25.79	2.39
(L.P.+G)	5	8.97	1.78	1.12	10.00	3.12	0.62	8.01	33.62	3.09
	6	5.12	1.15	0.67	4.19	1.76	0.42	4.44	17.75	0.90
	7	8.79	1.25	1.10	5.75	2.78	0.64	5.07	25.38	1.72
3 (L.P.+G)	2	6.65	0.97	0.32	7.04	2.98	1.23	4.68	23.87	3.61
	3	6.58	1.79	0.69	7.20	3.73	1.18	5.77	26.94	3.19
	4	9.32	1.11	0.82	6.18	3.96	0.90	4.69	26.98	2.47
(L.P.+M)	5	8.48	2.04	1.06	9.86	3.31	0.34	7.39	32.48	4.10
	6	7.28	1.45	1.03	6.60	3.08	0.44	4.36	24.24	2.23
	7	6.79	1.35	0.78	8.36	2.63	0.70	3.85	24.46	1.76
4 (H.P.)	2	5.67	1.03	0.50	6.98	2.67	1.60	4.32	22.77	2.89
	3	5.79	1.56	1.34	7.42	3.18	1.13	4.42	24.84	2.24
	4	4.87	1.00	0.69	5.33	2.91	0.95	3.71	19.46	2.28
(H.P.)	5	7.37	1.35	0.83	8.93	2.90	0.64	5.72	27.74	3.79
	6	6.19	1.03	1.00	6.12	2.51	0.35	4.24	21.44	3.17
	7	5.91	1.60	1.32	6.18	2.92	0.47	4.81	23.21	2.11

Table 28. Ratio of serum essential free amino acids to AIB in Experiment 3

Treatment[1]	Age wks	Serum essential free amino acids / AIB												
		Arg	Lys	His	Meth	Cys	Gly	Phe	Tyr	Leu	Iso	Thr	Val	Total
1 (L.P.)	2	2.54	4.63	0.45	0.38	1.24	1.69	0.74	0.94	1.30	0.85	1.38	1.05	17.19
	3	3.33	5.99	1.25	0.52	1.77	2.41	1.12	1.30	1.69	1.16	1.71	1.54	23.78
	4	3.11	3.34	0.63	0.42	1.61	2.17	0.93	1.37	1.65	0.75	1.13	1.09	18.23
(L.P.)	5	4.18	3.93	0.34	0.45	1.75	2.70	1.06	1.65	1.69	0.92	1.53	1.46	21.67
	6	5.03	5.11	1.34	0.74	1.75	2.61	1.35	2.97	2.09	1.46	2.08	1.97	28.51
	7	4.05	3.29	0.54	0.52	0.98	2.64	1.16	1.76	1.97	1.11	1.60	1.83	21.46
2 (L.P.+M)	2	2.31	4.20	0.28	0.31	1.15	1.37	0.68	0.78	1.12	0.70	1.09	0.97	14.98
	3	3.06	4.86	0.37	0.39	1.34	2.05	0.98	1.35	1.40	0.74	1.63	1.11	19.28
	4	3.89	4.11	0.85	0.49	2.05	2.16	1.22	1.39	2.33	1.03	1.05	1.82	22.39
(L.P.+G)	5	3.01	2.47	0.39	0.39	1.33	2.22	0.83	1.39	1.51	0.72	1.43	1.43	17.12
	6	8.14	8.91	0.99	1.24	2.23	4.40	2.04	2.94	3.44	2.31	3.98	2.72	43.37
	7	4.54	4.17	0.94	0.63	1.65	2.81	1.33	1.95	2.05	1.26	1.98	1.87	25.20
3 (L.P.+G)	2	2.74	4.02	0.43	0.26	1.18	1.56	0.80	0.99	1.04	0.67	1.01	0.83	15.53
	3	2.36	5.21	0.61	0.35	1.21	2.09	0.87	1.13	1.37	0.84	1.84	1.25	19.13
	4	3.13	3.25	0.34	0.43	1.65	2.24	1.06	1.20	1.63	0.86	1.73	1.54	19.06
(L.P.+M)	5	2.26	1.48	0.24	0.30	0.85	1.39	0.47	1.24	0.98	0.48	1.03	1.02	11.74
	6	3.89	3.17	0.41	0.60	1.57	1.92	1.02	1.44	1.76	0.86	1.54	2.83	21.03
	7	5.39	3.97	0.49	0.66	1.55	2.44	1.28	1.83	2.28	1.25	1.90	2.18	25.21
4 (H.P.)	2	4.11	7.61	0.42	0.41	1.76	1.74	1.07	1.12	1.99	1.06	1.85	1.34	24.47
	3	3.81	6.44	0.58	0.64	0.20	2.34	1.39	1.43	2.61	1.54	2.05	2.21	27.08
	4	3.02	3.67	0.39	0.41	1.75	1.83	1.07	1.49	1.90	0.95	1.29	1.53	19.29
(H.P.)	5	2.36	2.09	0.25	0.32	1.54	1.31	0.71	1.20	1.23	0.64	1.01	1.36	14.03
	6	1.98	1.85	0.27	0.37	0.99	1.30	0.67	1.03	0.90	0.66	1.03	1.16	12.23
	7	3.12	3.05	0.56	0.51	1.24	2.09	0.99	1.42	1.46	0.95	2.10	1.70	19.20

Table 29. Ratio of serum non-essential free amino acids to AIB in Experiment 3

Treatment[1]	Age wks	Serum non-essential free amino acids/AIB							
		Ale	Asp	HO-P	Glu	Pro	Orn	Ser	Total
1 (L.P.)	2	1.54	0.37	0.15	2.17	0.88	0.28	1.22	6.61
	3	2.45	0.60	0.37	2.96	1.37	0.46	1.82	10.03
	4	2.05	0.46	0.31	2.55	1.06	0.35	1.93	8.71
(L.P.)	5	3.33	0.63	0.29	0.37	1.31	0.26	2.25	8.44
	6	3.61	0.65	0.34	3.39	1.39	0.35	2.72	12.45
	7	3.94	0.70	0.68	3.17	1.56	0.29	2.87	13.21
2 (L.P.+M)	2	1.53	0.32	0.17	1.81	0.84	0.37	0.91	5.95
	3	2.76	0.54	0.30	2.64	1.13	0.35	1.98	9.70
	4	2.84	0.52	0.41	2.84	1.64	0.47	2.08	10.80
(L.P.+G)	5	2.90	0.58	0.36	3.24	1.01	0.20	2.59	10.88
	6	5.69	1.28	0.74	4.66	1.96	0.47	4.93	19.73
	7	5.11	0.73	0.64	3.34	1.62	0.37	2.95	14.76
3 (L.P.+G)	2	1.84	0.27	0.09	1.95	0.83	0.34	1.30	6.62
	3	2.06	0.56	0.22	2.26	1.17	0.37	1.81	8.45
	4	3.77	0.45	0.33	0.25	1.60	0.36	1.90	8.66
(L.P.+M)	5	2.07	0.50	0.26	2.40	0.81	0.08	1.80	7.92
	6	3.26	0.65	0.46	2.96	1.38	0.20	1.96	10.87
	7	3.86	0.77	0.44	4.75	1.49	0.40	2.19	13.90
4 (H.P.)	2	1.96	0.36	0.17	2.42	0.92	0.55	1.49	7.87
	3	2.58	0.70	0.60	3.31	1.42	0.50	1.97	11.08
	4	2.14	0.44	0.30	2.34	1.28	0.42	1.63	8.55
(H.P.)	5	1.94	0.36	0.22	2.36	0.77	0.17	1.51	7.33
	6	1.95	0.32	0.32	1.93	0.79	0.11	1.34	6.76
	7	2.80	0.76	0.63	2.93	1.38	0.22	2.28	11.00

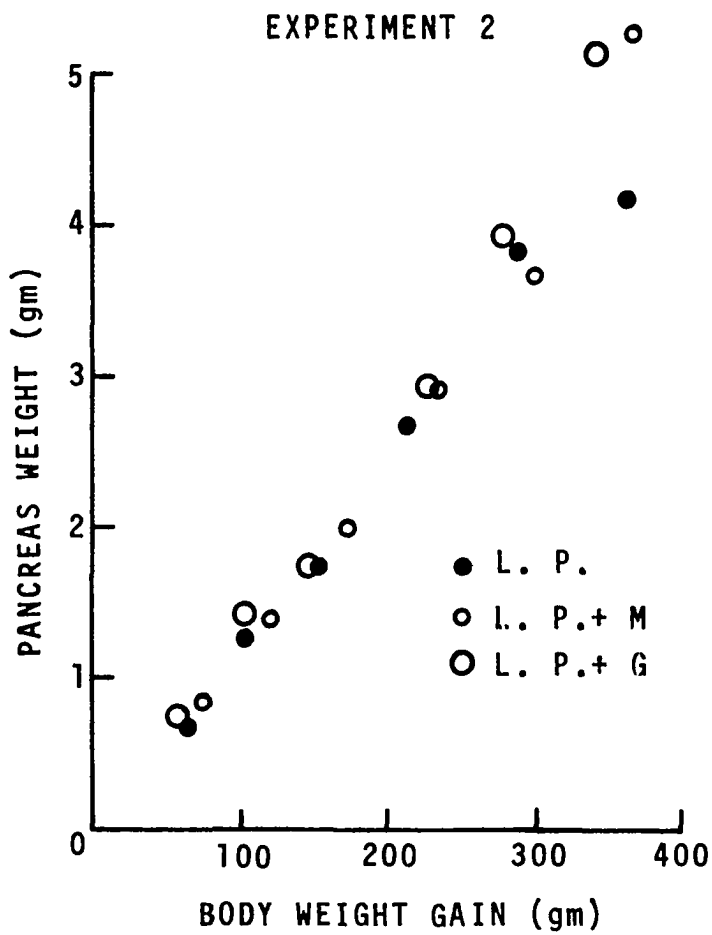
DISCUSSION

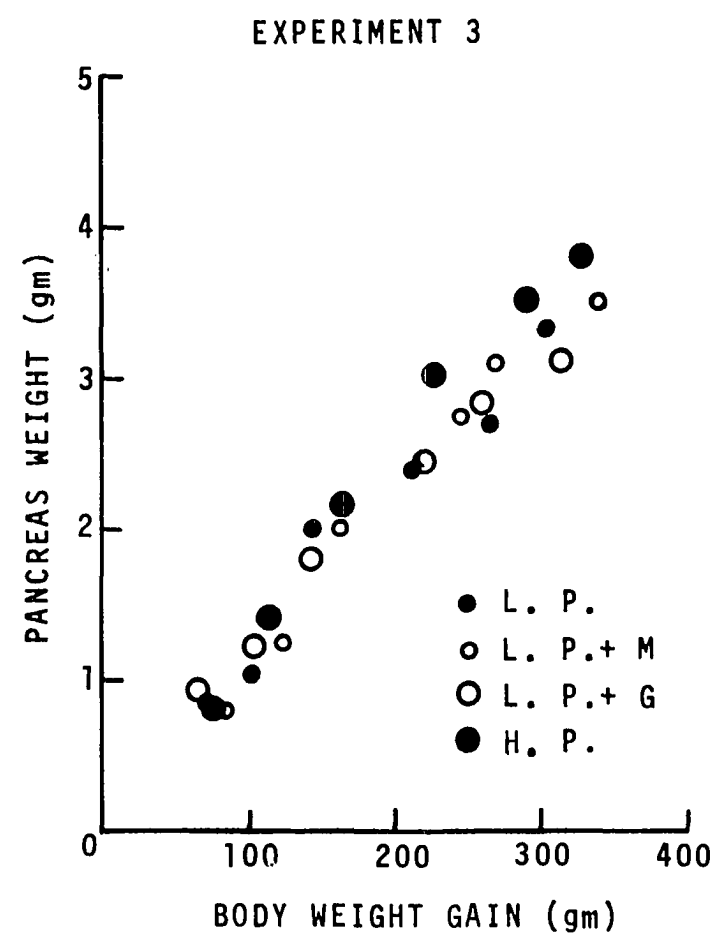
Body weight gain and feed efficiency were improved in three experiments by increasing dietary protein from 20 to 30% or by supplementing limiting amino acids to the basal diet. The benefit of improving the dietary amino acid balance appeared very effective in this case and could not be replaced by a non-essential amino acid such as glutamic acid.

Greater improvements in weight gains and feed efficiency were obtained in Experiment 1 when a 20% protein diet was supplemented with 0.3% arginine, 0.22% methionine and 0.6% lysine, and the 25% protein diet was supplemented with 0.11% methionine to reach National Research Council's requirement. In addition, the same improvements were repeated in Experiments 2 and 3 when 27% and 25% protein diets were supplemented with 0.11% of methionine. However, the supplementation of 0.11% of glutamic acid instead of methionine in the same diets caused no improvement in gain or feed conversion, showing that the limiting nutrient in the low-protein diet was not total nitrogen, per se. These results give complete support to the work of Balloun (1962; 1967), and Askelson and Balloun (1965).

As expected, the pancreas size increased with age. Without doubt, this effect was due to the high correlation between pancreas weight and body gain (Fig. 3). If true differences existed in pancreas size because of treatments, statistical significance could not be shown because of the few samples

Figure 3. Relationship of body weight gain and pancreas weight


EXPERIMENT 2
PANCREAS WEIGHT (gm)
BODY WEIGHT GAIN (gm)
0
1
2
3
4
5
100
200
300
400
L. P.
L. P.+ M
L. P.+ G


EXPERIMENT 3
PANCREAS WEIGHT (gm)
BODY WEIGHT GAIN (gm)
0
1
2
3
4
5
100
200
300
400
L. P.
L. P.+ M
L. P.+ G
H. P.

collected and the variation among individuals poults. Since the total soluble protein of the pancreas is a relatively fixed percentage of the pancreas, obviously, there are high correlations between total soluble protein and pancreas size (Fig. 4).

The most striking aspect of these trials is the discovery of the negative correlation between proteolytic enzymes specific activity (S.A) and total soluble protein of the pancreas (Fig. 5 and 6). In general, the specific activity decreased as total soluble protein increased. Furthermore, the pancreas of poults from the low-protein diet and low-protein with supplementation of glutamic acid treatments had higher specific activities than did the high-protein and the low-protein with supplementation of methionine treatments.

Nasset (1965), working with dogs, and Bird (1968) with chicks demonstrated that the amount of endogenous nitrogen in the gut lumen was suprisingly large. These reports lead to the question, are pancreatic proteins hydrolytic enzymes only, or are proteins other than hydrolytic enzymes secreted in the pancreas and discharged into the gut for homeostatic purposes? Our results, which favor the latter thought, showed a greater amount of pancreatic protein, but a less potent enzyme specific activity. This is thought to be because part of the proteins excreted by the pancreas are for homeostatic purposes; this portion of the protein may be varied by the dietary amino

Figure 4. Relationship of pancreas weight and total soluble protein

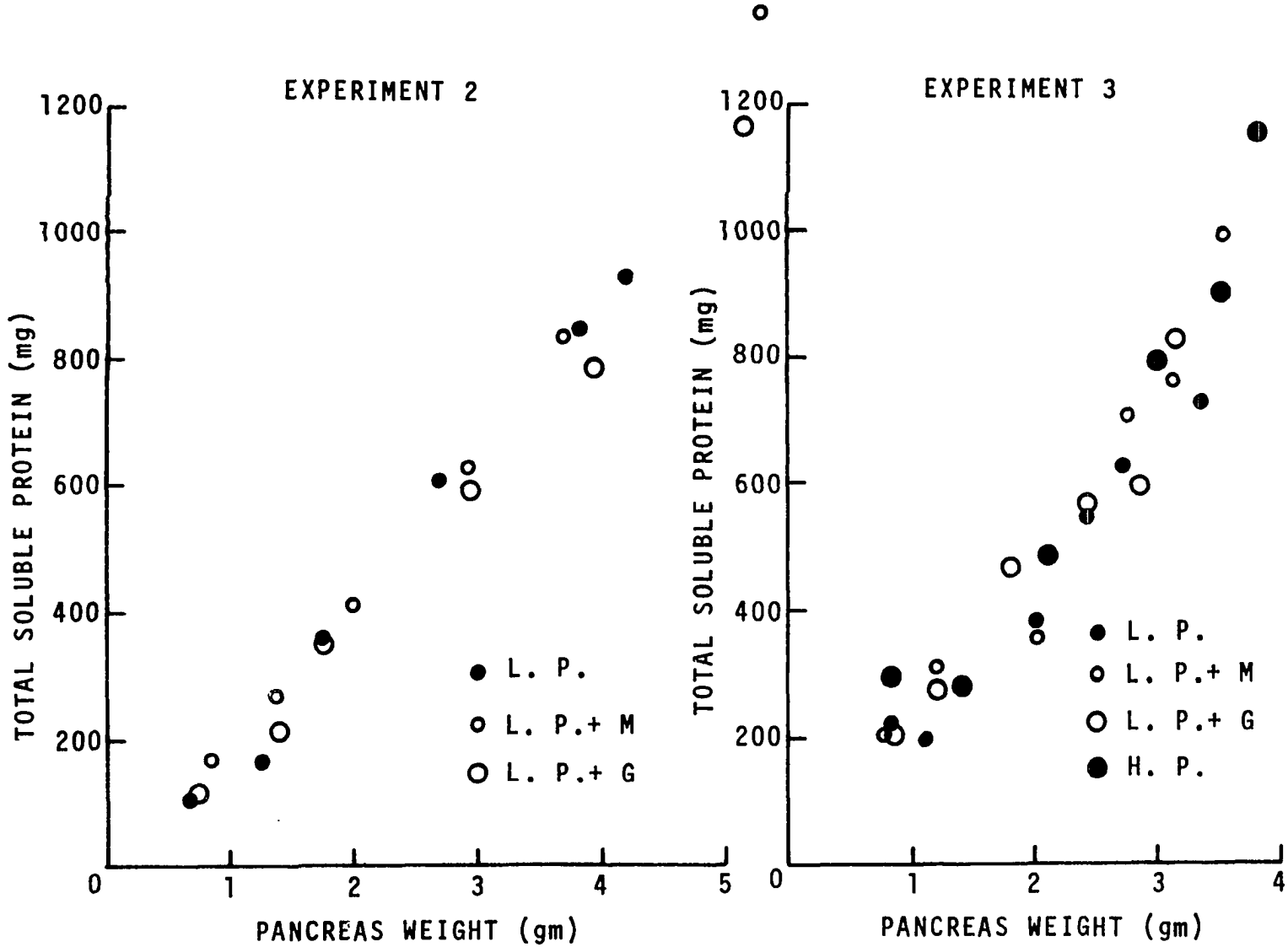

EXPERIMENT 2
TOTAL SOLUBLE PROTEIN (mg)
1200
1000
800
600
400
200
0
1
2
3
4
5
PANCREAS WEIGHT (gm)
L. P.
L. P.+ M
L. P.+ G
EXPERIMENT 3
TOTAL SOLUBLE PROTEIN (mg)
1200
1000
800
600
400
200
0
1
2
3
4
PANCREAS WEIGHT (gm)
L. P.
L. P.+ M
L. P.+ G
H. P.

Figure 5. Relationship of pancreas total soluble protein and protelytic enzyme specific activity (Experiment 2)

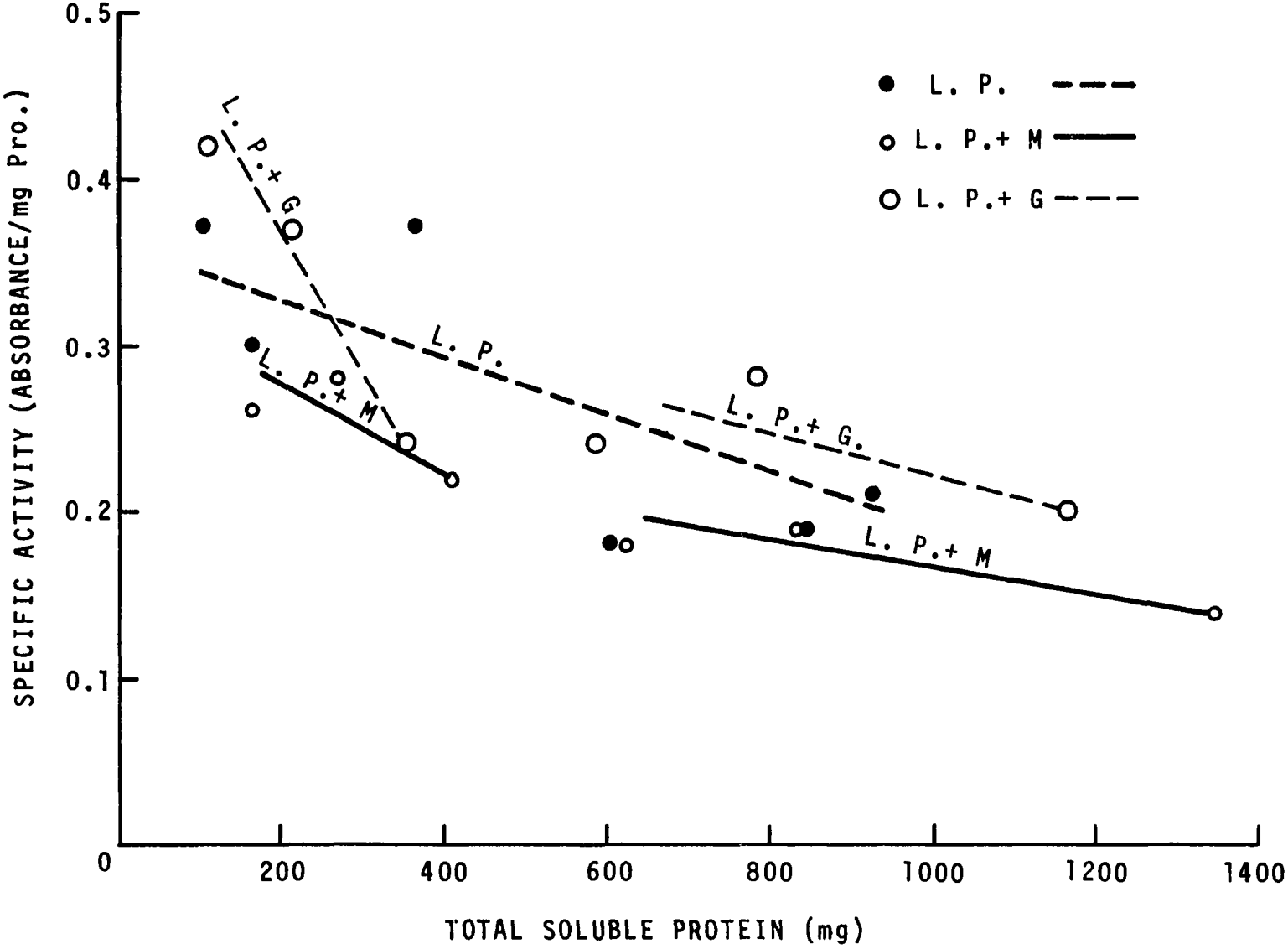
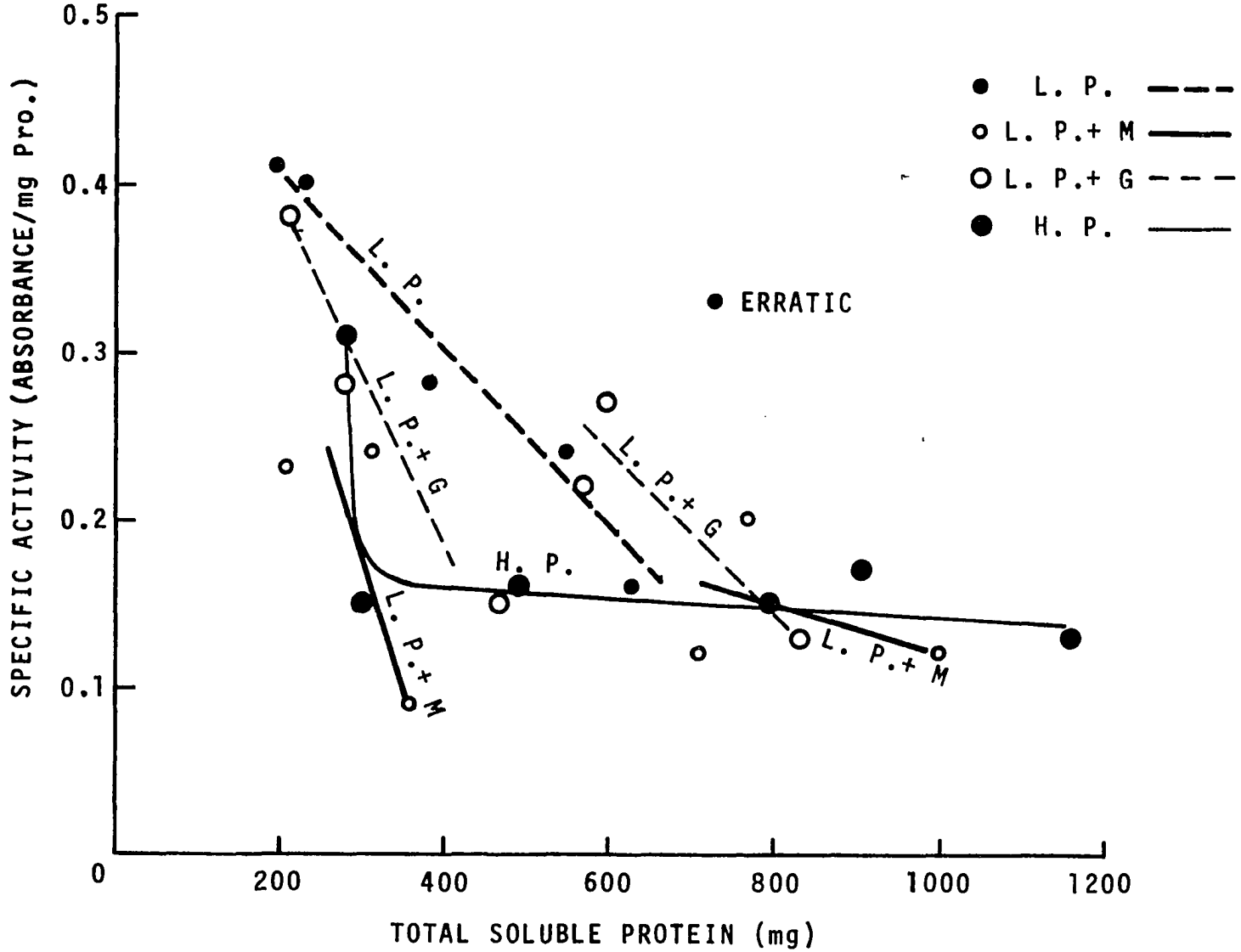

SPECIFIC ACTIVITY (ABSORBANCE/mg Pro.)
0.5
0.4
0.3
0.2
0.1
0
200
400
600
800
1000
1200
1400
TOTAL SOLUBLE PROTEIN (mg)
L. P.
L. P.+ M
L. P.+ G
L. P.+ G
L. P.
L. P.+ G.
L. P.+ M
L. P.+ M

Figure 6. Relationship of pancreas total soluble protein and protelytic enzyme specific activity (Experiment 3)


0.5
0.4
0.3
0.2
0.1
0
200
400
600
800
1000
1200
SPECIFIC ACTIVITY (ABSORBANCE/mg Pro.)
TOTAL SOLUBLE PROTEIN (mg)
L. P.
L. P.+ M
L. P.+ G
H. P.
ERRATIC
L. P.
L. P.+ G
L. P.+ G
H. P.
L. P.+ M
L. P.+ M

acid balance. Imondi and Bird (1967) reported that the pancreas contained proteases after 17 days of protein-free feeding, indicating that there was a continuous loss of non-proteolytic substances. These non-proteolytic substances very possibly may be the homeostatic proteins from the pancreas.

It has been demonstrated by numerous research works that liver plays an important role in the regulation of amino acid metabolism; therefore, an effect of nutritional status on liver response is expected. In Experiment 3 the effects of age, two levels of dietary protein and diet supplementation with crystalline amino acid on (1) the amount of DNA in liver tissue, (2) the liver DNA and RNA ratio, and (3) the percentage of liver protein were determined.

Currently it is commonly accepted that there is hypertrophy instead of hyperplasia in the liver of birds after hatch under normal nutritional status. The amount of DNA per gram of liver tissue demonstrates this aspect. From our results, the DNA densities appeared greater at the 4th than at the 7th week of age. This phenomenon is probably because the liver cells increased their cytoplasma at the later age, and consequently the DNA densities appeared low. Contrary to the hypertrophy theory, the balanced diets (high protein and low protein + methionine) stimulated a greater density of DNA in the liver than did the low protein diet at both 4 and 7 weeks

of age. Therefore, hyperplasia might still exist in poults in the early life stage after hatch. In addition, liver hyperplasia also was stimulated by glutamic acid supplementation at the 4th week of age. The liver probably provides more cell numbers in the unite of tissue, attempting to catabolize this extra compound.

It was unexpected that such a group of constant values were observed from RNA:DNA ratio. Two reasons are proposed to explain this. First of all, the levels of dietary protein and crystalline amino acids added to the diets may not have been large enough to stimulate the response of RNA:DNA ratio. Secondly, the 16 hours fast before the collection of liver samples might have depleted cell contents to a constant level.

No differences were found among treatment groups for liver protein at the 4th week of age. This is probably because the poults were too young and the metabolic responses were not sensitive enough to reflect the difference of nutritional status. However, at 7 weeks, liver protein percentages of poults fed balanced diets (high protein and low protein + methionine) were significantly greater than in poults fed methionine-deficient diets (low protein and low protein + glutamic acid). This is reasonable, because more amino acids should be absorbed from a balanced diet than from a methionine-deficient diet. There was a greater amount of amino

acids passing through the liver from the portal vein. consequently, liver enzymes (such as transaminase, deaminase and dehydrogenase) could be elevated.

The increased body weight gain observed in poults fed low-protein diets with the supplementation of limiting amino acids is becoming more important each day, since the cost of crystalline amino acids has been cheaper than in the past. However, the body gain should be re-evaluated by the parameter of body protein composition. Herein we hope the body protein composition is not adversely changed by the crystalline amino acids added to the diet.

Since Thomas and Combs (1967) suggested that serum protein or albumin levels may serve as an index of body protein composition, their suggestion was applied in this series of three experiments.

In Experiment 1, the levels of albumin increased linearly with the protein percentages in the diet. Poults from both treatments 2 and 4 (amino acid supplementation) had greater levels of albumin than poults from other treatments. Although there were no statistically significant differences observed among treatments, this is probably because the protein levels were close to a normal range.

In Experiments 2 and 3, serum albumin increased linearly with age. Older poults would be expected to have a higher body protein concentration than young poults in this period of

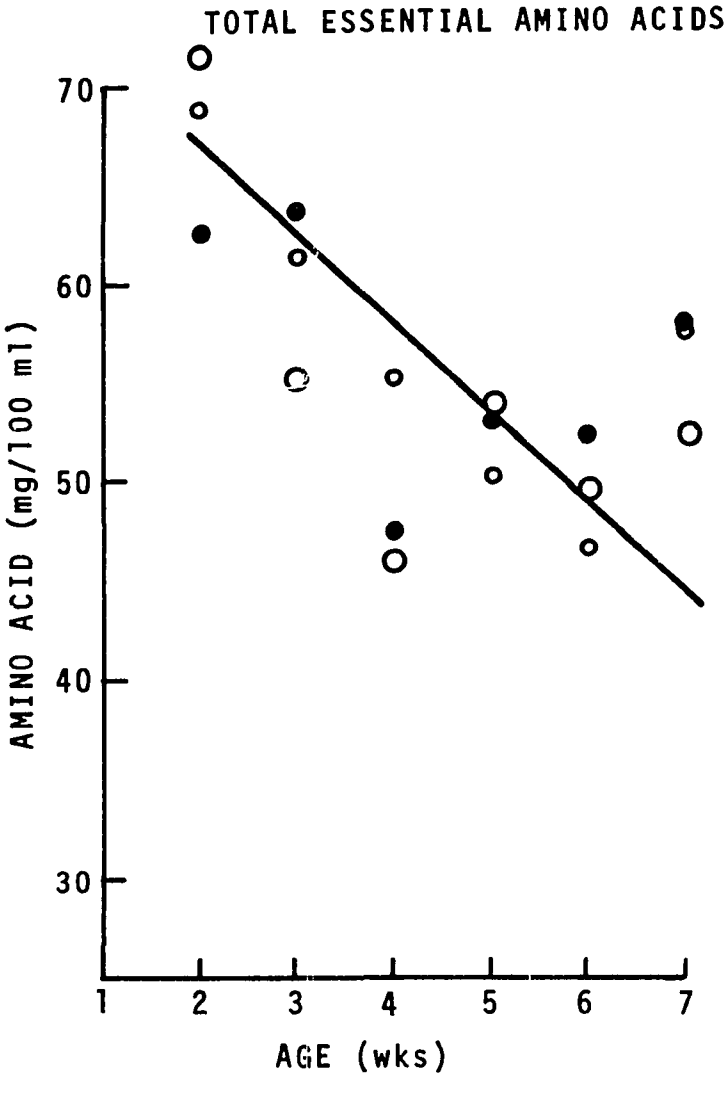
age. Among the treatments, there were no differences detected, except that the high-protein treatment tended to cause a slightly higher level of albumin than the low-protein treatment in Experiment 3. The conclusion could be made, therefore, that the supplementation of crystalline amino acids has no negative effect on body protein composition.

Serum protein concentration, contrary to serum albumin, was not correlated with age; furthermore, there were no trends observed among treatments. Serum protein level is probably not a good parameter to use for this kind of study. These results agree with Albanese (1959) that low-protein intakes might not be associated with low plasma protein level.

It is interesting to note that the total serum essential free amino acids decreased significantly with age in both Experiments 2 and 3 (Fig. 7 and 8), regardless of diet treatment. This phenomenon probably is due to the great importance of the temporal blood amino acid pool which acts as a body reservoir in the early life of the poult. When poults are older, the muscle is built up, consequently the amino acids of the body reservoir are shifted from blood to muscle amino acid pool. However, the total non-essential free amino acids in serum were much more constant.

Furthermore, this phenomenon of the bird's early life not only exists in respect to blood amino acid reservoir. It was

Figure 7. Relationship of age and serum total essential or non-essential free amino acids (Experiment 2)


TOTAL ESSENTIAL AMINO ACIDS
AMINO ACID (mg/100 ml)
70
60
50
40
30
1
2
3
4
5
6
7
AGE (wks)

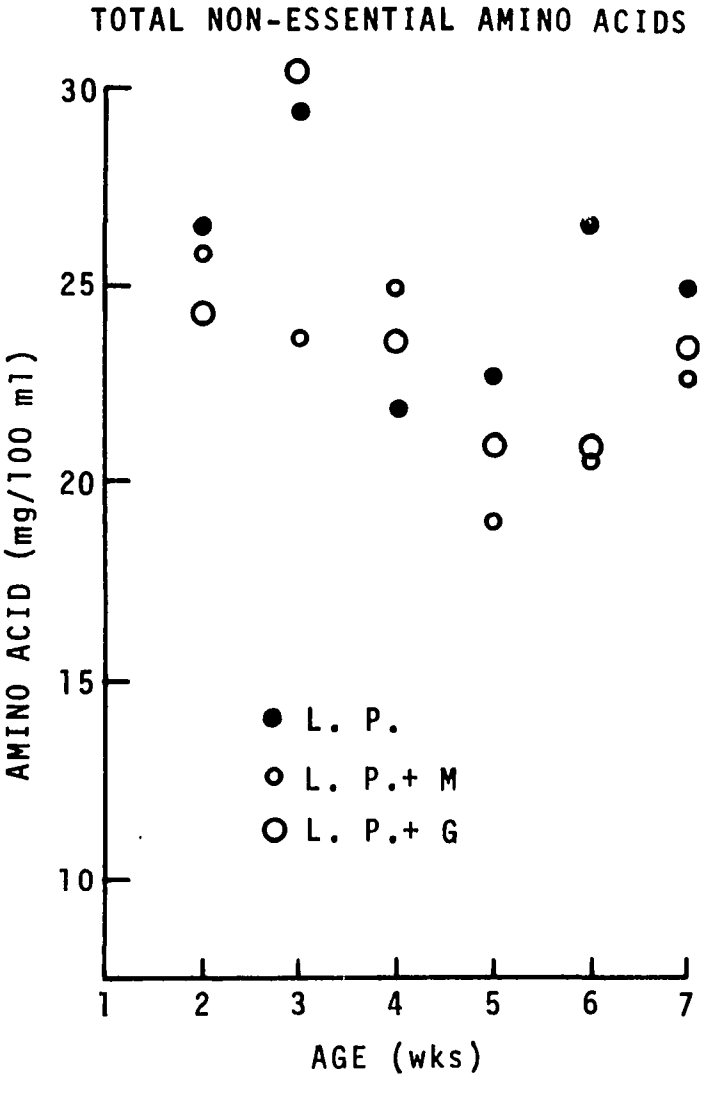
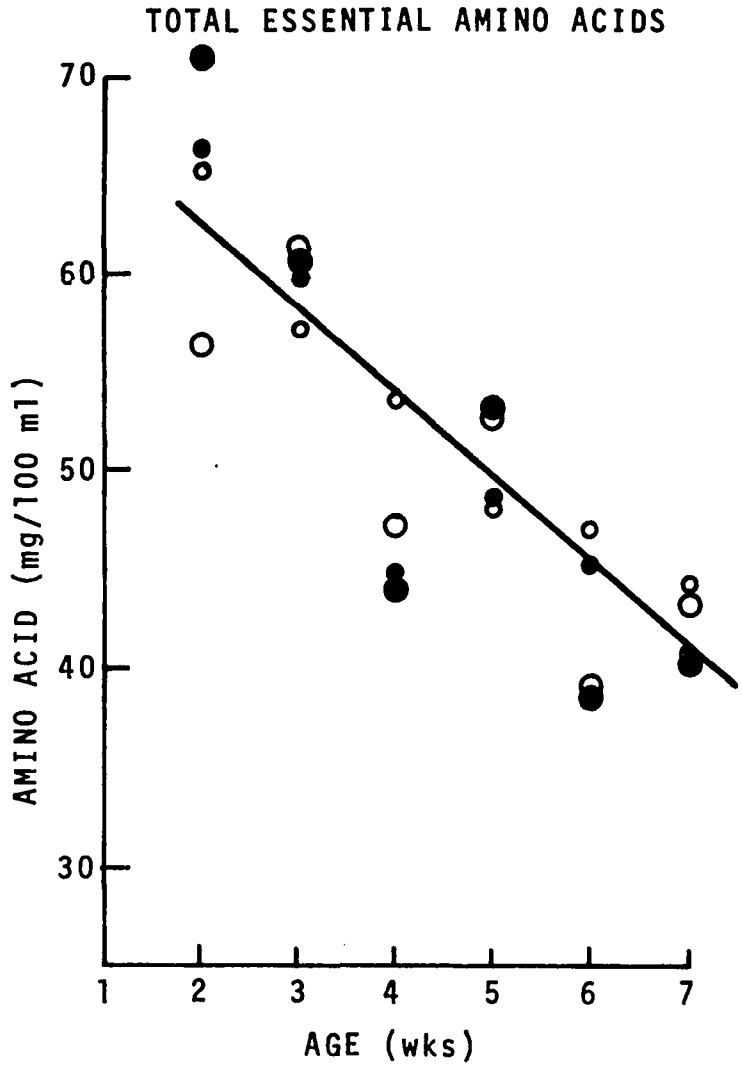
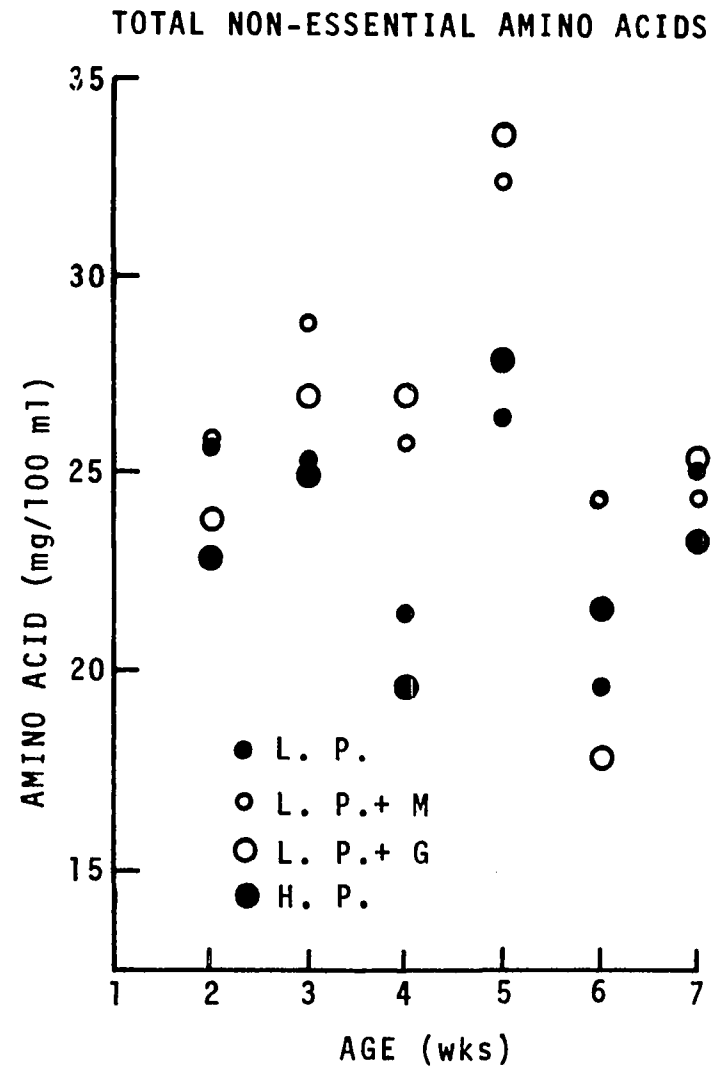

TOTAL NON-ESSENTIAL AMINO ACIDS
AMINO ACID (mg/100 ml)
30
25
20
15
10
L. P.
L. P.+ M
L. P.+ G
1
2
3
4
5
6
7
AGE (wks)

Figure 8. Relationship of age and serum total essential or non-essential free amino acids (Experiment 3)


TOTAL ESSENTIAL AMINO ACIDS
AMINO ACID (mg/100 ml)
70
60
50
40
30
1
2
3
4
5
6
7
AGE (wks)


TOTAL NON-ESSENTIAL AMINO ACIDS
AMINO ACID (mg/100 ml)
35
30
25
20
15
L. P.
L. P.+ M
L. P.+ G
H. P.
1
2
3
4
5
6
7
AGE (wks)

also found by Pratt and Terner (1971), when they studied the development of amino acid transport by the small intestine of the chick embryo. They observed that the amino acid active transport increased to a maximum by the day of hatch and declined rapidly thereafter until 3 weeks after hatching and then remained constant.

From the evidence, it is probably reasonable to conclude that free amino acids of the body reach an optimum level in the transport system, which includes absorption and circulation systems, at an early age in birds, probably by the day of hatching. The serum amino acid level declines as tissue protein synthesis and tissue amino acid reservoir develop. Therefore, the blood free amino acid level plays an important role in nutrition only at an early stage. Subsequently, the nutritional importance decreases as the tissue amino acid pool becomes stabilized.

Regardless of the significant body weight differences in gains among treatment groups, the serum free amino acids showed great consistency at any given age. Furthermore, the essential amino acid pattern in serum was very constant from 2 to 7 weeks of age, except for lysine, which had a more rapid declining rate. In general, this delicate homeostatic-control mechanism in the blood probably is due to three factors: (1) absorption could adjust the constant free amino acids in the blood, (2) excretion could stabilize blood free amino acids,

and (3) interchange of free amino acids between blood and tissue at a later life stage (after 3 or 4 weeks of age). Therefore, the true effect of dietary treatments on blood free amino acids would not show unless allowance had been made for these three factors.

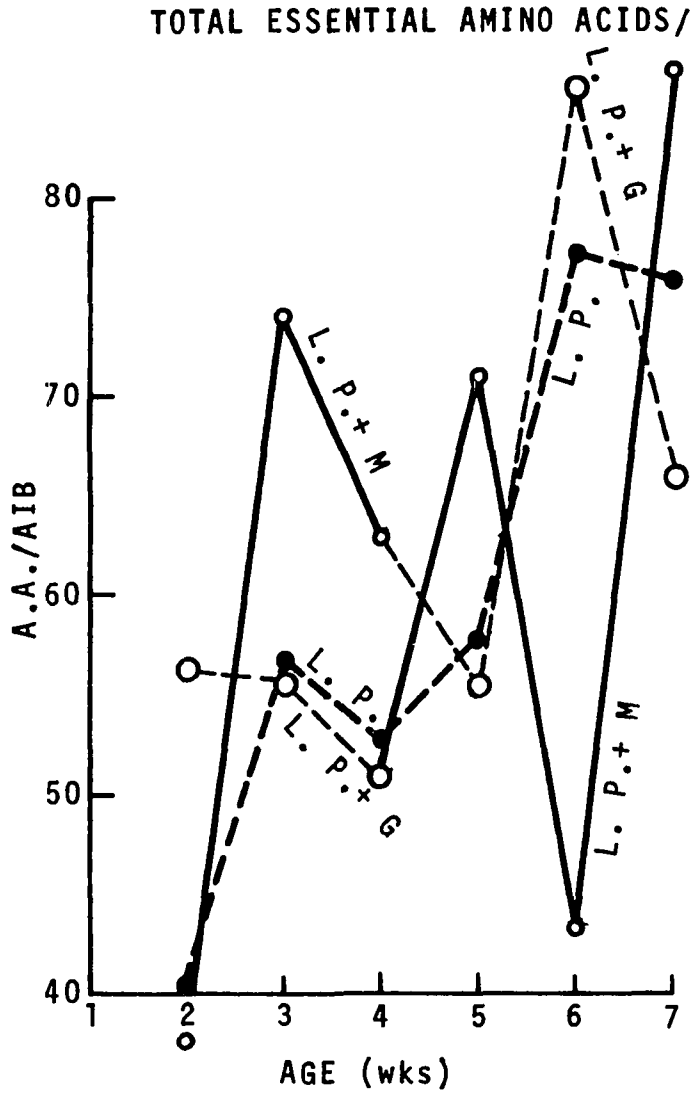
Since AIB has been used as a metabolic indicator very extensively for various purposes for amino acid studies, we measured the ratio of blood free amino acids to AIB in order to exclude the variation of absorption, excretion and interchange between blood and tissue.

Based on the results of Experiments 2 and 3, we have plotted means of the ratio of total essential and non-essential amino acids to AIB against age in weeks in Fig. 9 and 10. The statistical analysis of variance indicated that the main effects were between the high protein treatment and the rest. Therefore, as expected, no treatment difference were observed in Experiment 2 because of lacking treatment 4 (high protein) in this experiment.

In Experiment 3 (Fig. 10), the high protein treatment caused a higher blood amino acid level than the other treatments in period 1. However, the reverse was true in period 2; the interaction effect is clear.

This interaction effect would explain our results perfectly, if our previous speculated age effect theory was correct. For instance, there was 5% more of protein (or amino

Figure 9. Relationship of age and the ratio of serum total essential or non-essential free amino acids to AIB (Experiment 2)


TOTAL ESSENTIAL AMINO ACIDS/AIB
A.A./AIB
80
70
60
50
40
1
2
3
4
5
6
7
AGE (wks)
L. P.
L. P.+ M
L. P.+ G

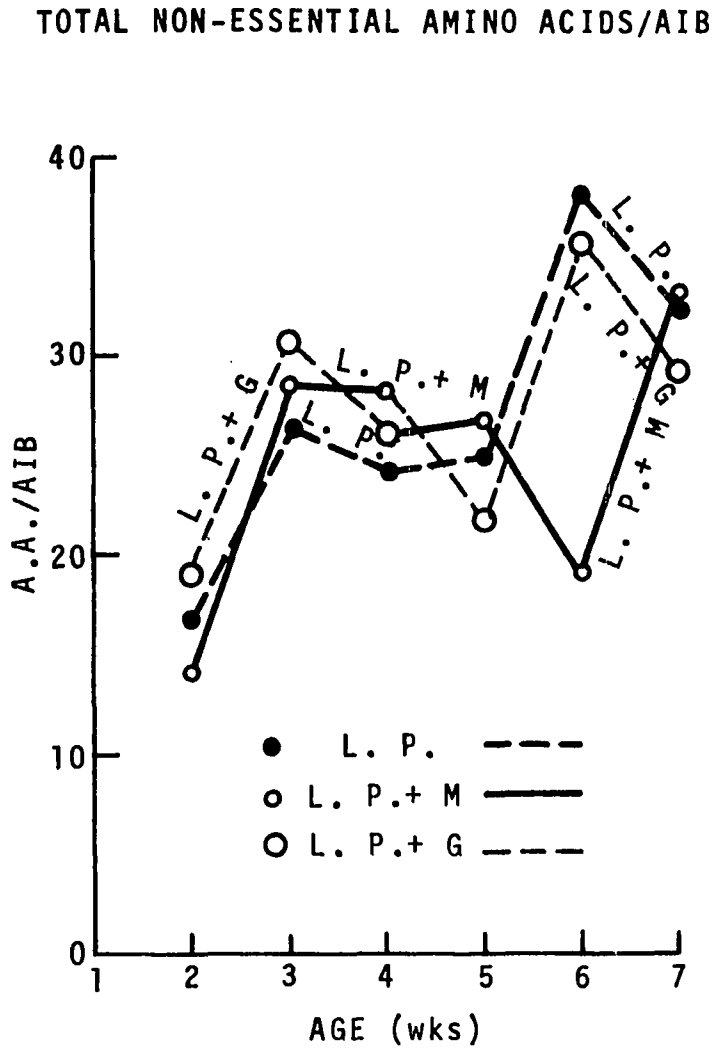
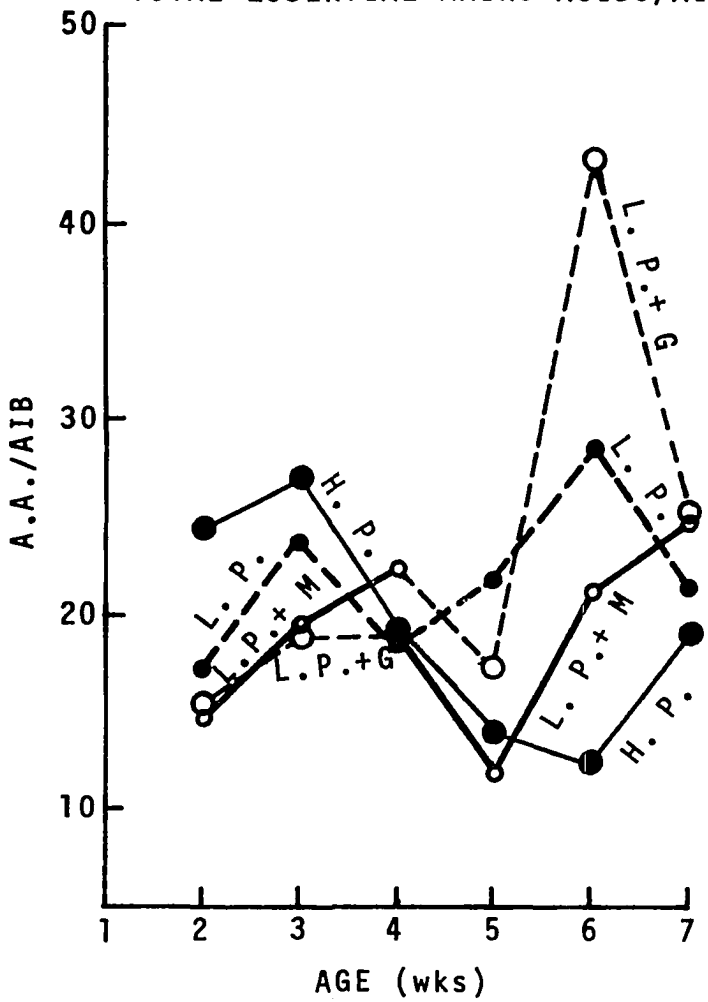
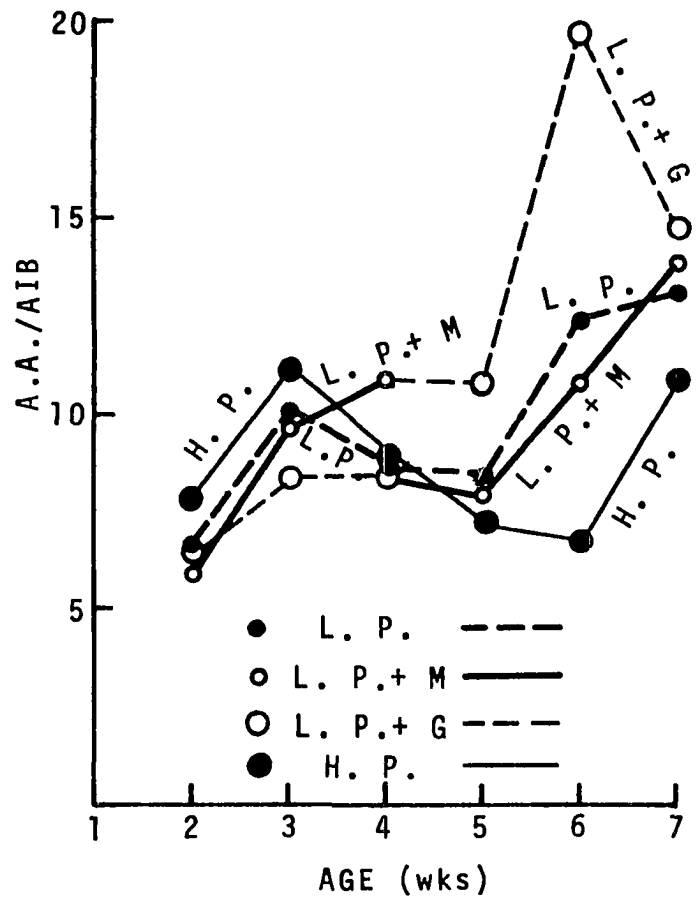

TOTAL NON-ESSENTIAL AMINO ACIDS/AIB
A.A./AIB
40
30
20
10
0
1
2
3
4
5
6
7
AGE (wks)
L. P.
L. P.+ M
L. P.+ G

Figure 10. Relationship of age and the ratio of serum total essential or non-essential free amino acids to AIB (Experiment 3)

TOTAL ESSENTIAL AMINO ACIDS/AIB



TOTAL NON-ESSENTIAL AMINO ACIDS/AIB

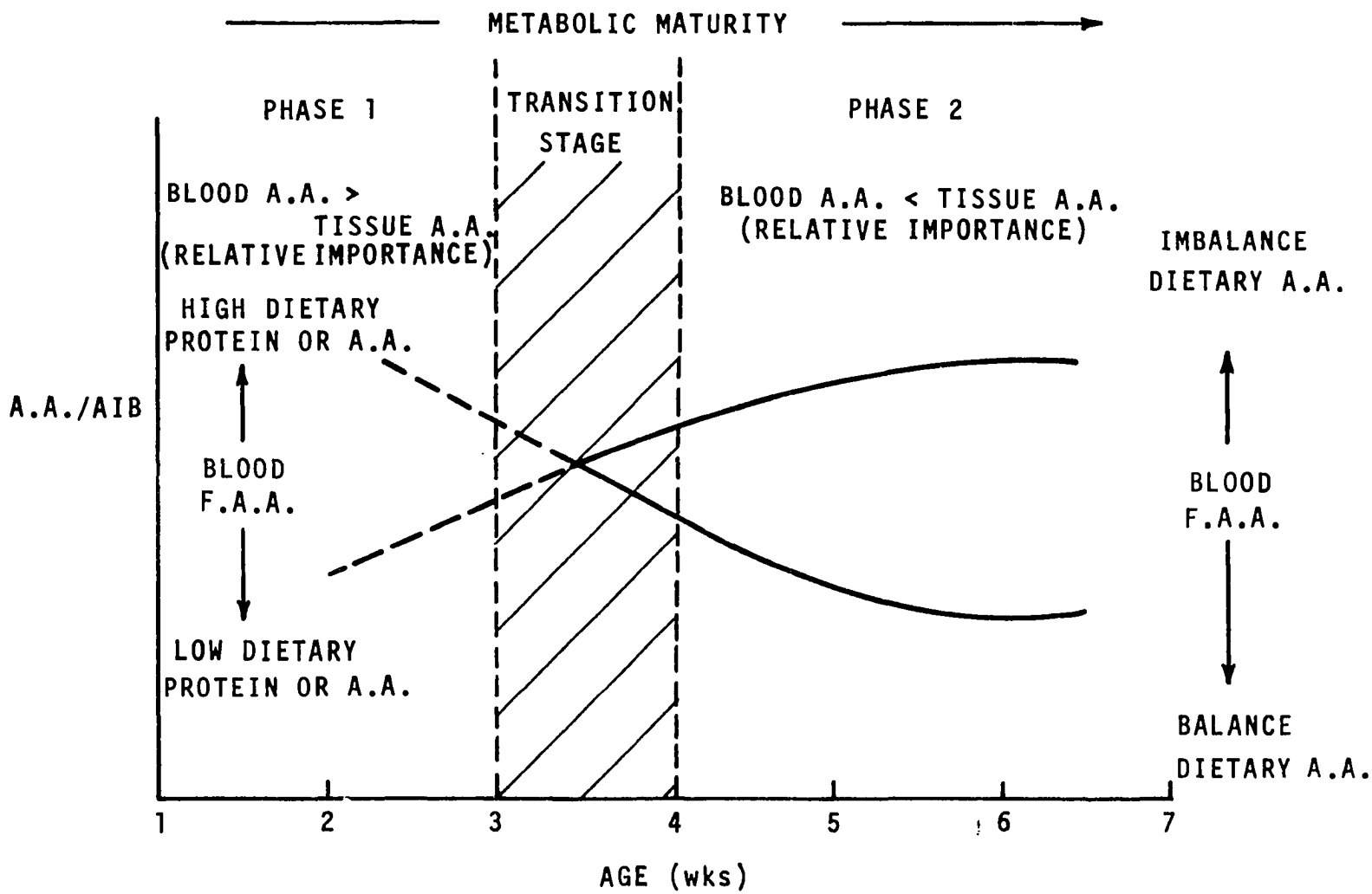


acids) in the diet of the high-protein treatment than in the other diets. Therefore, in period 1, the poults fed the high-protein diet had higher amino acid levels in the blood. Since the muscle amino acid pool was not yet established in period 1, the blood amino acid pool was the only reservoir to reflect the magnitude of total amount of amino acids or protein in the diet. However, later in period 2, as muscle protein synthesis and the muscle amino acid pool built up, the blood amino acid level of poults fed the high-protein diet, which had a better balance of dietary amino acids, was rapidly transformed into the muscle amino acid pool. Subsequently, the blood amino acid level decreased sharply. Comparatively, the poults fed the methionine-deficient diet (low protein and low protein + glutamic acid) had a higher blood amino acid level than those fed the high-protein diet. This was due to the poor balance of dietary amino acids limiting the influx to the muscle amino acid pool. In period 2, the poults from the methionine-supplemented treatment tended to approach the high-protein treatment in their blood amino acids pattern. However, since the approachment was not complete, the second limiting amino acid (such as lysine or arginine) may be the reason for restriction of blood amino acid transport into the muscle pool.

In summary and conclusion of all of these results, we are encouraged to propose a complete picture of the total blood

amino acid level by the interaction effect of dietary amino acid balance and age in Fig. 11. In phase 1, which is the early life of poults (from hatch to approximately 3 weeks of age) the blood essential amino acid level depends on the amount of protein or amino acids in the diet. In this stage, because the blood amino acid pool is the only reservoir, the amount of dietary protein or amino acids fed, the greater the blood amino acid level observed. In phase 2, the tissue amino acid pool has been established, and the balanced amino acids move into the tissue pool from the blood more than from a deficient or imbalanced amino acids. Therefore, in this stage, the birds fed a diet with balanced amino acids show a lower blood essential amino acid level than do the birds fed a diet deficient or imbalanced in amino acids.

Figure 11. Reflection of total blood free amino acids (amino acids/AIB) level by the interaction effect of dietary amino acids and age


METABOLIC MATURITY
PHASE 1
TRANSITION STAGE
PHASE 2
BLOOD A.A. > TISSUE A.A. (RELATIVE IMPORTANCE)
BLOOD A.A. < TISSUE A.A. (RELATIVE IMPORTANCE)
IMBALANCE DIETARY A.A.
HIGH DIETARY PROTEIN OR A.A.
A.A./AIB
BLOOD F.A.A.
BLOOD F.A.A.
LOW DIETARY PROTEIN OR A.A.
BALANCE DIETARY A.A.
1
2
3
4
5
6
7
AGE (wks)

SUMMARY AND CONCLUSIONS

Three experiments were conducted to investigate the effect of protein levels and limiting amino acid(s) supplementation on metabolic responses in male turkey poults. The basic protein sources for the turkey starter diets were conventional corn-soybean diets. Methionine, which was the first limiting amino acid in the basal diets, arginine and lysine were used for the supplementation.

The criteria of metabolic responses were (1) body weight gain and feed efficiency, (2) pancreas size, total soluble protein and enzyme specific activity, (3) liver DNA density, RNA to DNA ratio and liver protein, (4) serum protein and albumin, and (5) serum free amino acids and ratio of free amino acids to AIB. The following have been concluded from the data obtained under the environmental and dietary conditions employed:

1. The supplementation of limiting amino acid(s) to low-protein diets increased body weight gain and feed conversion to equal those obtained with a high-protein diet.
2. Pancreas size and pancreatic total soluble protein increased linearly with body weight gain. The pancreatic proteolytic enzyme specific activity decreased as pancreatic total soluble protein increased. Furthermore, the methionine-limiting

treatments caused higher specific activities than did the dietary balanced treatments when the correlation was made between specific activity and total soluble protein of the pancreas.

3. Liver DNA densities appeared greater at the 4th than at the 7th week of age, the phenomenon being due to hypertrophy. However, the amino acid balanced treatments stimulated a greater density of DNA in the liver than did the low-protein treatment at both the 4th and 7th week of age. Therefore, it is concluded that hyperplasia might still exist in poults in early life. In addition, liver hyperplasia was affected by glutamic acid supplementation into the low-protein diet at the 4th week of age. Liver protein percentage of poults fed amino acid balanced diets was significantly greater than in poults fed a methionine-deficient diet at 7 weeks of age.
4. Serum albumin tended to increase with age and with increased dietary protein. There was no change in serum albumin when amino acid(s) were supplemented, consequently, the supplementation of crystalline amino acids had no negative effect on body protein composition. Regardless of age or treatment differences, serum protein of poults appeared very constant.

5. Total serum free amino acids decreased with age; lysine had a more rapid decreasing rate than did other amino acids. Regardless of the significant body weight differences in gains among treatment groups, the serum free amino acids showed consistency at any given age. However, when serum free amino acids were measured by ratio to AIB, the interaction effects due to age and treatments were observed. Therefore, the great importance of the temporal blood amino acid pool, which acts as a body reservoir in the early life of the poults, is suggested. In addition, the magnitude of free amino acids in serum is a reflection of the amount of dietary protein in poults' early life (approximate 3 to 4 weeks of age). After 4 weeks of age, the serum free amino acid levels are dominated by the balanced status of dietary amino acid composition.
6. Adding glutamic acid to the basal low-protein diet did not result in gains and other metabolic responses equal to those of poults fed a basal diet with methionine supplementation, indicating that the depressed gains were due to a methionine deficiency and not to total nitrogen supplied.

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Sincere appreciation is expressed to my wife for her help in conducting the experiments, collecting data and typing this thesis.

The author is very grateful to Mrs. Balloun for her kindness like a mother; this spiritual support is invaluable.

APPENDIX

Table 30. Analysis of variance for poult 3 weeks body weight gain in Experiment 1

Source	d.f.	Mean square
Treatment	4	3277.64**
Error	10	165.45

Duncan's Multiple-Range Test ($S_{\bar{x}}$ = 7.4)

Value of P	2	3	4	5
SSR	3.15	3.30	3.37	3.43
LSR	23.31	24.42	24.94	25.38

206.3	255.4	258.7	283.3	290.5

**Probability = 0.01 or less, here and throughout.

Table 31. Analysis of variance for poult 3 weeks feed efficiency in Experiment 1

Source	d.f.	Mean square
Treatment	4	0.0276**
Error	10	0.0033

Duncan's Multiple-Range Test ($S_{\bar{x}}$ = 0.03)

Value of P	2	3	4	5
SSR	3.15	3.30	3.37	3.43
LSR	0.095	0.099	0.101	0.103

1.47	1.50	1.51	1.58	1.71

Table 32. Analysis of variance for poult 5 weeks body weight gain in Experiment 1

Source	d.f.	Mean square
Treatment	4	13723.38**
Error	10	873.37

Duncan's Multiple-Range Test ($S_{\bar{x}}$ = 17.1)

Value of P	2	3	4	5
SSR	3.15	3.30	3.37	3.43
LSR	53.9	56.4	57.6	58.7

500.73	594.19	643.70	655.72	663.80

Table 33. Analysis of variance for poult 5 weeks feed efficiency in Experiment 1

Source	d.f.	Mean square
Treatment	4	0.0530**
Error	10	0.0038

Duncan's Multiple-Range Test ($S_{\bar{x}}$ = 0.04)

Value of P	2	3	4	5
SSR	3.15	3.30	3.37	3.43
LSR	0.126	0.132	0.135	0.137

1.83	1.88	1.95	2.00	2.18

Table 34. Analysis of variance for poult body weight gain and feed efficiency in Experiment 2

Source	d.f.	Mean square	
		Gain/Poult	F.Intake/Gain
Treatment	2		
(A) T_1, T_3 vs T_2	1	219.7	0.0080
(B) T_1 vs T_3	1	138.3	0.0148
Error (Pen/Treatment)	9	650.1	0.0308
Week	5		
(A) 2,3,4 vs 5,6,7	1	574640.0**	2.4496**
(B) Linear (Period 1)	1	50828.0**	0.1472**
(C) Quadratic (Period 1)	1	51.4	0.0012
(D) Linear (Period 2)	1	104243.0**	0.7072**
(E) Quadratic (Period 2)	1	24.2	0.0116
Treatment x Week	10		
TA x WA	1	3786.4**	0.4140**
TA x WB	1	116.8	0.0160
TA x WC	1	14.1	0.0280
TA x WD	1	726.3*	0.0640*
TA x WE	1	48.6	0.0012
TB x WA	1	781.5*	0.0800*
TB x WB	1	0.4	0.0036
TB x WC	1	35.6	0.0644*
TB x WD	1	180.1	0.0144
TB x WE	1	0.1	0.0004
Error	45	141.6	0.0128

*Probability = 0.05 or less, here and throughout.

Table 35. Analysis of variance for pancreas weight, pancreas total soluble protein and enzyme specific activity in Experiment 2

Source	d.f.	Mean square		
		Pancreas wt.	Sol. Protein	S.A.
Treatment	2			
(A) T_1, T_3 vs T_2	1	0.5828	5100.5	0.0002
(B) T_1 vs T_3	1	0.6256	60139.4	0.0012
Error (Pen/Treatment)	9	0.1819	22252.3	0.0100
Week	5			
(A) 2,3,4 vs 5,6,7	1	114.7104**	6752784.0**	0.2788**
(B) Linear (Period 1)	1	6.8264**	377754.8**	0.0096
(C) Quadratic (Period 1)	1	0.0356	3029.0	0.0004
(D) Linear (Period 2)	1	24.6444**	1746897.8**	0.0024
(E) Quadratic (Period 2)	1	0.0128	24383.6	0.0072
Treatment x Week	10			
TA x WA	1	0.0660	19390.7	0.0504
TA x WB	1	0.0160	16.3	0.0243
TA x WC	1	0.0452	160.4	0.0001
TA x WD	1	0.1120	3201.4	0.0002
TA x WE	1	0.0144	5980.6	0.0049
TB x WA	1	0.3136	60991.6	0.0021
TB x WB	1	0.0036	144.0	0.0361
TB x WC	1	0.0084	147.0	0.0176
TB x WD	1	0.7396	158006.2*	0.0049
TB X WE	1	0.8112	72852.3	0.0016
Error	45	0.1331	26778.0	0.0140

Table 36. Analysis of variance for serum albumin in Experiment 2

Source	d.f.	Mean square Serum Albumin
Treatment	2	
(A) T_1, T_3 vs T_2	1	0.8997
(B) T_1 vs T_3	1	2.4474
Error (Pen/Treatment)	9	28.9900
Week	5	
(A) 2,3,4 vs 5,6,7	1	242.5862**
(B) Linear (Period 1)	1	16.6664
(C) Quadratic (Period 1)	1	24.4998
(D) Linear (Period 2)	1	53.6404
(E) Quadratic (Period 2)	1	14.1157
Treatment x Week	10	
TA x WA	1	11.4466
TA x WB	1	11.9601
TA x WC	1	0.0169
TA x WD	1	66.8350
TA x WE	1	13.1536
TB x WA	1	2.8617
TB x WB	1	41.8605
TB x WC	1	1.4282
TB x WD	1	64.0000
TB x WE	1	12.1603
Error	45	25.9800

Table 37. Analysis of variance for serum lysine, glycine and threonine in Experiment 2

Source	d.f.	Mean square		
		Lysine	Glycine	Threonine
Treatment	2			
(A) T_1, T_3 vs T_2	1	24.9004	1.6900	3.8284
(B) T_1 vs T_3	1	0.0040	3.7184	5.2536
Error (Pen/Treatment)	9	13.1443	2.6733	2.6646
Week	5			
(A) 2,3,4 vs 5,6,7	1	628.2328**	30.0056**	20.4800**
(B) Linear (Period 1)	1	315.6644**	46.4816*	16.8004**
(C) Quadratic (Period 1)	1	3.1084	1.9868	0.4356
(D) Linear (Period 2)	1	3.6192	1.0584	1.8372
(E) Quadratic (Period 2)	1	5.4012	1.7924	0.0016
Treatment x Week	10			
TA x WA	1	5.7440	1.4080	0.7920
TA x WB	1	30.2104	0.3604	1.9360
TA x WC	1	0	1.6728	0.0692
TA x WD	1	2.6132	2.5392	0.0832
TA x WE	1	12.2032	0.0348	2.8448
TB x WA	1	10.5656	0.1044	10.7920
TB x WB	1	92.9296*	1.4400	0.0024
TB x WC	1	124.4204**	0.7700	0.0120
TB x WD	1	6.9696	0.0784	1.3456
TB x WE	1	1.6724	0.3072	0.6912
Error	45	16.3185	1.5294	2.0923

Table 38. Analysis of variance for serum glutamic acid and the ratio of threonine to AIB in Experiment 2

Source	d.f.	Mean square	
		Glutamic Acid	Thr/AIB
Treatment	2		
(A) T_1, T_3 vs T_2	1	0.1320	3.0508
(B) T_1 vs T_3	1	1.7480	9.6480
Error (Pen/Treatment)	9	5.1018	4.0018
Week	5		
(A) 2,3,4 vs 5,6,7	1	43.8052**	11.2968
(B) Linear (Period 1)	1	48.3936**	2.4068
(C) Quadratic (Period 1)	1	6.8696	19.2200*
(D) Linear (Period 2)	1	8.0272	0.0524
(E) Quadratic (Period 2)	1	4.6004	8.9324
Treatment x Week	10		
TA x WA	1	0.2208	1.8676
TA x WB	1	0.0972	5.7684
TA x WC	1	9.9436	6.1504
TA x WD	1	0.1008	2.2532
TA x WE	1	2.0832	0.3288
TB x WA	1	10.0468	28.2132*
TB x WB	1	0.0256	0.0900
TB x WC	1	0.1540	1.0560
TB x WD	1	0.6888	11.2896
TB x WE	1	10.1936	62.3808**
Error	45	4.1651	4.6570

Table 39. Analysis of variance for poult body weight gain and feed efficiency in Experiment 3

Source	d.f.	Mean square	
		Gain/Poult	F. Intake/Gain
Treatment	3		
(A) T_1, T_3 vs T_2, T_4	1	2689.2*	0.0384
(B) T_1 vs T_3	1	1786.0	0.0056
(C) T_2 vs T_4	1	1334.0	0.0320
Error (Pen/Treatment)	12	490.4	0.0136
Week	5		
(A) 2,3,4 vs 5,6,7	1	632960.8**	3.8081**
(B) Linear (Period 1)	1	49798.4**	0.3528**
(C) Quadratic (Period 1)	1	54.8	0.0001
(D) Linear (Period 2)	1	80200.0**	0.4608**
(E) Quadratic (Period 2)	1	259.6	0.0216
Treatment x week	15		
TA x WA	1	584.4	0.3083**
TA x WB	1	209.2	0.0008
TA x WC	1	13.6	0.0001
TA x WD	1	432.0	0.0722
TA x WE	1	32.0	0.0001
TB x WA	1	1678.4*	0.0280
TB x WB	1	20.8	0.0064
TB x WC	1	180.8	0.0225
TB x WD	1	16.0	0.0025
TB x WE	1	1140.4	0.0147
TC x WA	1	3154.0**	0.1008*
TC x WB	1	129.6	0.0256
TC x WC	1	15.2	0
TC x WD	1	786.8	0.0529
TC x WE	1	75.6	0.0040
Error	60	338.4	0.0225

Table 40. Analysis of variance for pancreas weight, pancreas total soluble protein and enzyme specific activity in Experiment 3

Source	d.f.	Mean square		
		Pancreas wt.	Sol. Pro.	S.A.
Treatment	3			
(A) T_1, T_3 vs T_2, T_4	1	0.2731*	72986.9	0.1121**
(B) T_1 vs T_3	1	0.1323	170049.8**	0.1083**
(C) T_2 vs T_4	1	1.7787**	371009.2**	0.0027
Error (Pen/Treatment)	12	0.0657	23879.2	0.0170
Week	5			
(A) 2,3,4 vs 5,6,7	1	72.1063**	5054028.0**	0.1176**
(B) Linear (Period 1)	1	10.3058**	284446.0*	0.1152*
(C) Quadratic (Period 1)	1	0.2817	43904.0	0.0726*
(D) Linear (Period 2)	1	6.1250**	609131.2**	0.0002
(E) Quadratic (Period 2)	1	0.0323	47481.3	0.0054
Treatment x Week	15			
TA x WA	1	0.0081	14826.2	0.0641
TA x WB	1	0.0648	2467.5	0.0289
TA x WC	1	0.0267	5118.7	0.0150
TA x WD	1	0.0968	11819.6	0.0162
TA x WE	1	0.1734	4746.0	0.0193
TB x WA	1	0.1680	56239.6	0.0003
TB x WB	1	0.0441	11502.6	0.0100
TB x WC	1	0.0456	2867.5	0.0048
TB x WD	1	0.1849	11556.3	0.0100
TB x WE	1	0.1160	7500.0	0.0589
TC x WA	1	0.8216*	147409.9	0.0176
TC x WB	1	0.0121	1560.3	0.0196
TC x WC	1	0.0096	27360.7	0.0065
TC x WD	1	0.0225	10251.6	0.0036
TC x WE	1	0.0016	1463.0	0.0065
Error	60	0.0901	27870.9	0.0165

Table 41. Analysis of variance for serum albumin in Experiment 3

Source	d.f.	Mean square Serum Albumin
Treatment	3	
(A) T_1, T_3 vs T_2, T_4	1	173.6**
(B) T_1 vs T_3	1	0.8
(C) T_2 vs T_4	1	0.7
Error (Pen/Treatment)	12	25.5
Week	5	
(A) 2,3,4 vs 5,6,7	1	536.0**
(B) Linear (Period 1)	1	181.4**
(C) Quadratic (Period 1)	1	0
(D) Linear (Period 2)	1	536.2**
(E) Quadratic (Period 2)	1	136.2*
Treatment x Week	15	
TA x WA	1	5.7
TA x WB	1	5.6
TA x WC	1	4.6
TA x WD	1	7.0
TA x WE	1	41.5
TB x WA	1	12.4
TB x WB	1	1.6
TB x WC	1	0.2
TB x WD	1	46.2
TB x WE	1	1.1
TC x WA	1	0.4
TC x WB	1	20.3
TC x WC	1	102.6**
TC x WD	1	18.6
TC x WE	1	2.3
Error	60	19.3

Table 42. Analysis of variance for serum lysine, Cystine and glycine in Experiment 3

Source	d.f.	Mean square		
		Lysine	Cystine	Glycine
Treatment	3			
(A) T_1, T_3 vs T_2, T_4	1	7.7	3.1828	3.2560
(B) T_1 vs T_3	1	8.4	0.0048	0.3400
(C) T_2 vs T_4	1	0	1.8408	8.2336*
Error (Pen/Treatment)	12	8.6	0.9251	1.3784
Week	5			
(A) 2,3,4 vs 5,6,7	1	1101.3**	33.3704**	12.8480**
(B) Linear (Period 1)	1	730.8**	2.5540	4.3512
(C) Quadratic (Period 1)	1	33.1	0.9600	3.8880
(D) Linear (Period 2)	1	6.3	26.7912**	12.5000**
(E) Quadratic (Period 2)	1	0.1	3.6192	13.9840**
Treatment x Week	15			
TA x WA	1	8.7	0.0072	2.3564
TA x WB	1	17.7	0.0200	0.0760
TA x WC	1	40.1	0.3360	0.0048
TA x WD	1	0	1.3780	0.0048
TA x WE	1	2.1	5.1524*	0.0728
TB x WA	1	0.3	1.6132	0.2296
TB x WB	1	10.0	0.3968	1.1236
TB x WC	1	14.4	0.2080	1.2288
TB x WD	1	12.0	1.6640	0.1368
TB x WE	1	0	0.3960	0.6816
TC x WA	1	7.7	2.7456	0.1324
TC x WB	1	26.5	1.0200	0.0008
TC x WC	1	1.7	1.3736	0.0208
TC x WD	1	1.0	3.8024	2.2200
TC x WE	1	4.9	0.1876	2.3408
Error	60	15.3	0.9153	1.4758

Table 43. Analysis of variance for serum phenylalanine, leucine and isoleucine in Experiment 3

Source	d.f.	Mean square		
		Phenylalanine	Leucine	Iso.
Treatment	3			
(A) T_1, T_3 vs T_2, T_4	1	0.4648	2.9540	0.4004
(B) T_1 vs T_3	1	0.0012	0	0.5376
(C) T_2 vs T_4	1	0.0016	0.1280	0.3468
Error (Pen/Treatment)	12	0.2078	2.0069	0.3558
Week	5			
(A) 2,3,4 vs 5,6,7	1	7.7292**	21.1688**	6.1408**
(B) Linear (Period 1)	1	1.1704*	0.9112	5.1864**
(C) Quadratic (Period 1)	1	0.2204	0.0004	0.7212
(D) Linear (Period 2)	1	0.2664	3.7812	0.0220
(E) Quadratic (Period 2)	1	0.4320	4.4892	0.0228
Treatment x Week	15			
TA x WA	1	0.0988	5.9400	0.0620
TA x WB	1	0.0060	0.0012	0.0288
TA x WC	1	0.0008	0.1504	0.1732
TA x WD	1	0.5512	3.6180	0.3280
TA x WE	1	0.5220	1.4308	0.0704
TA x WA	1	0.1120	1.8408	0.0560
TA x WB	1	0.0900	1.5128	1.3224
TA x WC	1	0.0832	0.8216	0.0076
TA x WD	1	0.2304	0	0.0484
TA x WE	1	0.1540	0.1876	0.1824
TA x WA	1	0.0508	1.3332	0.2944
TA x WB	1	0.3720	4.4100	0.0840
TA x WC	1	0.2080	4.5632	2.5576
TA x WD	1	0.1088	0.1936	0.1368
TA x WE	1	0.1496	0.0012	0
Error	60	0.2519	1.2302	0.3143

Table 44. Analysis of variance for serum threonine and ornithine in Experiment 3

Source	d.f.	Mean square	
		Threonine	Ornithine
Treatment	3		
(A) T_1, T_3 vs T_2, T_4	1	0.3752	0.3268
(B) T_1 vs T_3	1	0.0032	0.0384
(C) T_2 vs T_4	1	0.3008	0.3604
Error (Pen/Treatment)	12	0.9017	0.1298
Week	5		
(A) 2,3,4 vs 5,6,7	1	8.1200*	12.4992**
(B) Linear (Period 1)	1	21.3856**	0.7936**
(C) Quadratic (Period 1)	1	7.3924*	0.3084
(D) Linear (Period 2)	1	1.3944	0.0164
(E) Quadratic (Period 2)	1	1.4504	0.1804
Treatment x Week	15		
TA x WA	1	1.3444	0.3552
TA x WB	1	3.7540	0.1152
TA x WC	1	0.0008	0.6272
TA x WD	1	0.3612	0.1152
TA x WE	1	0.5104	0.0728
TB x WA	1	2.1336	0.0012
TB x WB	1	10.1124*	0.0440
TB x WC	1	1.3600	0.0096
TB x WD	1	0.2500	0.1520
TB x WE	1	0.2132	0.0056
TC x WA	1	0.1408	0.1280
TC x WB	1	0.0324	0.8280**
TC x WC	1	0.8320	0.3816
TC x WD	1	2.7224	0.0360
TC x WE	1	0.3676	0.0004
Error	60	1.4449	0.1008

Table 45. Analysis of variance for the ratio of serum arginine, lysine and methionine to AIB in Experiment 3

Source	d.f.	Mean square Arg/AIB	Lys/AIB	Meth/AIB
Treatment	3			
(A) T_1, T_3 vs T_2, T_4	1	2.9680	14.6016	0.1472
(B) T_1 vs T_3	1	3.1828	9.6840	0.0800
(C) T_2 vs T_4	1	14.3008*	5.5760	0.1324
Error (Pen/Treatment)	12	2.9885	4.2878	0.0576
Week	5			
(A) 2,3,4 vs 5,6,7	1	35.2352**	19.8744	0.9440**
(B) Linear (Period 1)	1	0.7936	21.9124	0.0648
(C) Quadratic (Period 1)	1	0.0352	25.9584	0.0772
(D) Linear (Period 2)	1	15.2904**	11.1864	0.4512**
(E) Quadratic (Period 2)	1	31.1448**	51.3336	1.2788*
Treatment x Week	15			
TA x WA	1	0.3084	0.0728	0.0384
TA x WB	1	0.0580	1.6200	0
TA x WC	1	0.1012	3.5576	0.0044
TA x WD	1	0.0112	1.9800	0.0112
TA x WE	1	9.8560*	8.5680	0.1980*
TB x WA	1	0.1084	1.4008	0.0148
TB x WB	1	0.0016	0.9800	0.0256
TB x WC	1	0.5808	0.1776	0.0084
TB x WD	1	9.9224*	12.3200	0.0676
TB x WE	1	1.1164	1.7788	0.0384
TC x WA	1	27.3008**	46.8076**	0.3816**
TC x WB	1	5.9536*	10.5624	0.0256
TC x WC	1	0.0900	0.0120	0.0644
TC x WD	1	2.0164	3.1684	0.0288
TC x WE	1	7.8732*	16.6144*	0.0736
Error	60	1.4815	3.5533	0.0298

Table 46. Analysis of variance for the ratio of serum glycine, phenylalanine and leucine to AIB in Experiment 3

Source	d.f.	Mean square Gly/AIB	Phe/AIB	Leu/AIB
Treatment	3			
(A) T_1, T_3 vs T_2, T_4	1	0.4988	0.9128	3.3004
(B) T_1 vs T_3	1	2.7648*	0.3200	0.6912
(C) T_2 vs T_4	1	4.9408**	0.3888	0.9296
Error (Pen/Treatment)	12	0.6225	0.2021	0.8773
Week	5			
(A) 2,3,4 vs 5,6,7	1	7.0632**	0.8068*	1.5708
(B) Linear (Period 1)	1	1.8432*	0.4800	1.5840
(C) Quadratic (Period 1)	1	1.5404*	0.2320	0.3552
(D) Linear (Period 2)	1	2.9040**	1.7672**	3.1752**
(E) Quadratic (Period 2)	1	4.6640**	2.4576**	3.9044**
Treatment x Week	15			
TA x WA	1	1.1880*	0.0452	0.3700
TA x WB	1	0.0096	0.0072	0.0648
TA x WC	1	0.0416	0.0068	0.0024
TA x WD	1	0.2520	0.0008	0.0576
TA x WE	1	4.2168**	0.4932	1.9040*
TB x WA	1	0.7300	0.1632	0.0032
TB x WB	1	0.0624	0.0120	0.1224
TB x WC	1	0.1680	0.1008	0.0056
TB x WD	1	1.0200	0.6724*	1.1448
TB x WE	1	0.0120	0.0048	0.0408
TC x WA	1	5.3068**	1.4700**	7.9056**
TC x WB	1	0.3968	0.2208	1.1024
TC x WC	1	0.0936	0.0868	0.9296
TC x WD	1	0.0196	0.2116	0.4760
TC x WE	1	0.4640	0.0768	1.1164
Error	60	0.2955	0.1249	0.4118

Table 47. Analysis of variance for the ratio of serum isoleucine, threonine and alanine to AIB in Experiment 3

Source	d.f.	Mean square Iso/AIB	Thr/AIB	Ala/AIB
Treatment	3			
(A) T_1, T_3 vs T_2, T_4	1	1.0416	2.0416	1.0836
(B) T_1 vs T_3	1	0.6720	0.7200	0.0384
(C) T_2 vs T_4	1	0.2820	0.5124	14.5640*
Error (Pen/Treatment)	12	0.2514	0.6539	2.0567
Week	5			
(A) 2,3,4 vs 5,6,7	1	1.2880**	4.9684**	44.5536**
(B) Linear (Period 1)	1	0.0180	0.0840	8.6112**
(C) Quadratic (Period 1)	1	0.4988	2.3688*	0.3900
(D) Linear (Period 2)	1	2.0000**	3.2512**	15.2352**
(E) Quadratic (Period 2)	1	3.2856**	7.7748**	6.7416**
Treatment x Week	15			
TA x WA	1	0.1120	1.7712	0.8588
TA x WB	1	0.0180	0.3280	0.3612
TA x WC	1	0.0120	0.0072	1.2788
TA x WD	1	0.0200	0.4324	0.5620
TA x WE	1	1.0252*	2.7880**	2.1840
TB x WA	1	0.0384	0.0012	2.7264
TB x WB	1	0.1444	1.6384*	2.1316
TB x WC	1	0.0588	1.1040	2.4300
TB x WD	1	0.3364	0.3248	1.0200
TB x WE	1	0.3200	0.3816	0.1496
TC x WA	1	2.8420**	5.6032**	7.2076*
TC x WB	1	0.1368	0.1680	1.5128
TC x WC	1	0.6256	0.0096	0.0456
TC x WD	1	0.3600	0.1296	4.1208*
TC x WE	1	0.3888	3.7632**	0.9296
Error	60	0.1624	0.3571	0.8309

Table 48. Analysis of variance for the ratio of serum aspartic acid and serine to AIB in Experiment 3

Source	d.f.	Mean square Asp/AIB	Mean square Ser/AIB
Treatment	3		
(A) T_1, T_3 vs T_2, T_4	1	0.1120	2.6400
(B) T_1 vs T_3	1	0.0456	1.4560
(C) T_2 vs T_4	1	0.3008*	7.4576**
Error (Pen/Treatment)	12	0.0415	0.7908
Week	5		
(A) 2,3,4 vs 5,6,7	1	1.5200**	23.9200**
(B) Linear (Period 1)	1	0.1624*	3.6992**
(C) Quadratic (Period 1)	1	0.3800**	1.1268
(D) Linear (Period 2)	1	0.3960**	2.6220**
(E) Quadratic (Period 2)	1	0.3128**	6.5104**
Treatment x Week	15		
TA x WA	1	0.0268	1.9268*
TA x WB	1	0	0
TA x WC	1	0.0072	0.3084
TA x WD	1	0.0544	0.0544
TA x WE	1	0.3128**	4.5936**
TB x WA	1	0.0176	0.7600
TB x WB	1	0.0048	0.0324
TB x WC	1	0.0004	0.0084
TB x WD	1	0.0324	0.2116
TB x WE	1	0	0.0108
TC x WA	1	0.3676**	6.1348**
TC x WB	1	0.0144	1.3456
TC x WC	1	0.0384	0
TC x WD	1	0.0288	0
TC x WE	1	0.3008**	1.7176*
Error	60	0.0362	0.3704